Federation Proceedings

VOLUME 8 *March 1949* NUMBER 1

AMERICAN PHYSIOLOGICAL SOCIETY

Fifty-eighth Annual Meeting

DETROIT, MICHIGAN, APRIL 18–22, 1949

(For possible corrections in any of the following abstracts see the June issue)

Experimental mitral insufficiency. W. E. ADAMS, E. B. BAY and M. M. NEWMAN (introduced by Lester R. Dragstedt). *Dept. of Medicine and Surgery, Univ. of Chicago, Chicago, Ill.* Recent revival of interest in surgical treatment of valvular heart disease has prompted study of the effects of pure mitral insufficiency. Thirty dogs were operated by transthoracic approach and the anterior mitral leaflet was cut with a hooked knife introduced through the left auricular appendage. Each dog was studied 4 to 5 times preoperatively at intervals of 1 to 2 months, determinations being made of cardiac output (Fick) and right ventricular and pulmonary artery pressures by right heart catheterization. Tracings were also made on a low-frequency, critically damped ballistocardiograph. Nineteen animals survived the immediate operative period, and 10 have survived 8 to 18 months to provide the present studies. All dogs have loud systolic murmurs and the heart size has increased appreciably on fluoroscopy, but no gross evidences of heart failure or obvious limitation of physical activity have developed. Cardiac output and systemic blood pressure have remained essentially unchanged. Right ventricular pressure has been elevated in most dogs to 30–40 mm. Hg systolic. In 4 of the 10 dogs, diastolic pressure has risen to 5–10 mm. Hg. Left auricular pressure was palpably increased at time of operation, but could not be measured in the intact animal. Contrary to expectations, the ballistocardiograph waves have shown no change in contour, and the correlation with direct Fick outputs showed no constant change. It would appear that those animals which survived operation by 2 weeks or more show no abnormality at the present time other than moderate pulmonary hypertension.

Adrenalectomy and renin proteinuria in the rat. T. ADDIS and ROBERT I. BOYD (by invitation). *Dept. of Medicine, Stanford Univ. Medical School, San Francisco, Calif.* In the rat the intraperitoneal injection of 4 mgm. of renin, with the pressor value of 1 U/mg., induces a variable but pronounced increase in the protein content of the urine. This increase quickly rises to a maximum and in a few hours is gone. A unique feature of this proteinuria is the excretion of fibrinogen, for in short time urine collections made soon after an injection there may form a fine network of what seems to be fibrin. Immediately after adrenalectomy, however, no proteinuria is induced by renin, and for 10 days after adrenalectomy rats maintained on sodium chloride and glucose with added B vitamins give no definite increase in protein excretion after renin. Attempts to reestablish the renin effect in adrenalectomized rats by the injection of massive doses of cortical extracts and of desoxycorticosterone acetate gave only uncertain and equivocal results. Several adrenalectomized rats responded with slight augmentation of protein excretion after renin, but these performances were not confirmed by group averages. Intermittent injection intraperitoneally of adrenalin in maximum tolerated dosage at short intervals failed to reestablish the renin effect in adrenalectomized animals. In 2 normal rats the administration of 2,4-methylpiperadyl benzodioxane (Fournand's 993) prior to the renin did not prevent nor appreciably alter the proteinuria response to renin.

Pulsatile evaporative rates from small skin areas as measured by an infra-red gas analyzer. R. E. ALBERT and E. D. PALMES (introduced by A. D. KELLER). *Medical Dept. Field Research Laby., Fort Knox, Ky.* In the course of thermal balance studies, the question arose as to why the sweat rate, as measured from the whole man by an infrared gas analyzer, was pulsatile. As an approach to the problem, the original method (Palmes. *Rev. Sci. Instr.* 19: 711, 1948) was modified so that measurements could be taken from relatively small skin areas. This was accomplished

by passing dry oxygen through a cup placed on the skin and then conducting the gas to the analyzer, where the concentration of the water vapor in the oxygen was determined. In this manner, continuous quantitative measurements of the evaporative rate were obtained from skin areas measuring about 20 cm^2. It was found that at low sweat rates, the pulses appear in bursts. At moderate rates, they occur continuously with an average frequency of 6–7 per minute, and at very high evaporative levels, both the frequency and amplitude of the pulsations are damped. The fluctuations in the evaporative rate, as determined by this method, are dependent on the variations in the amount of skin area that is wet. The 90% response time of the instrument, which is 8 seconds, limits the maximum frequency of change that can be detected. Therefore, higher frequencies of change of rate may exist. Using duplicate sets of equipment, the measurement of dissimilar, actively sweating skin areas demonstrates that the rate variations occur simultaneously. This indicates that most of the active glands are discharged at the same time, possibly by a common stimulus arising centrally.

Serum prothrombic activity and prothrombin conversion accelerator in hemophilia and thrombocytopenia. BENJAMIN ALEXANDER and GRETA LANDWEHR (introduced by HERRMAN L. BLUMGART). *Medical Research Laby., Beth Israel Hospital and Dept. of Medicine, Harvard Medical School, Boston, Mass.* The prothrombic activity (1-stage) of normal human serum averages 6%. Serum and plasma mixtures exhibit more activity (faster prothrombin conversion) than the sum of the components. This is referable to a conversion accelerator (spca) which evolves during coagulation, and which can be adsorbed with $BaSO^4$ and eluted with citrate. Sera from thrombocytopenic and hemophilic blood, or blood clotted slowly in silicone, show abnormally high prothrombic activity, in hemophilia often exceedingth at of the parent plasma. Also, they cannot accelerate thrombin evolution from added prothrombin. Nevertheless, adsorption and elution of such sera yields fractions poorer in prothrombin and capable of accelerating prothrombin conversion of added prothrombin preparations. The agent can thus, in the one stage method, accelerate thrombin formation from unconsumed prothrombin in these sera, interpretable as a much higher prothrombin concentration than actually obtains. That these sera cannot accelerate conversion of additional prothrombin supplied as plasma or prothrombin fractions suggests that spca is linked to unconsumed prothrombin. By the 2-stage method, which does not measure velocity, it would have no effect. This may explain the discrepant results obtained on hemophilic serum with both procedures. Furthermore, prothrombin consumption in the coagulation of hemophilic or thrombocytopenic blood computed from differences between plasma and serum prothrombic activities (1-stage) are erroneous. Spca can be separated in a fraction containing 20 mg. protein from 100 cc. of serum. Many physiological and biochemical properties have been delineated. It appears different from serum Ac-globulin since the latter is said to be remarkably unstable in human serum whereas spca is relatively stable.

Femoral arterial pulse in aortic insufficiency. ROBERT S. ALEXANDER. *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, Ohio.* Aortic insufficiency was produced acutely in anesthetized dogs while recording pressure pulses from the femoral artery with optical manometers. When these recordings were analyzed and compared with similar recordings obtained under other experimental conditions it was found that; *1*) the high peak of systolic pressure in the femoral artery represents an augmentation of the large central aortic pulse pressure that is relatively no greater than that observed normally. *2*) The sharp drop from this sytolic peak does not represent aortic regurgitation, but is simply a consequence of the high systolic level attained; relatively the fall in pressure is slower than normal. *3*) The only abnormal change in pulse form produced by aortic insufficiency is the disappearance of the dicrotic waves. The latter is attributed to the elimination of the aortic standing wave, primarily because the creation of an opening at the central end of the aorta during diastole destroys the resonant properties of this system. It is therefore concluded that the Corrigan pulse observed in aortic insufficiency can be explained simply in terms of present concepts of pulse dynamics without the necessity of any additional assumptions.

Changes in the clotting mechanism produced by protamine sulfate and heparin. J. GARROTT ALLEN and WILLADENE EGNER (by invitation). *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* Changes produced in the whole blood clotting time of the normal dog are fairly standard when 0.5 mg/kg. body weight of heparin is administered intravenously. The normal clotting time is spontaneously returned to normal 90 to 120 minutes after heparin injection. The whole blood clotting time was returned to normal within 3 minutes when 0.5 mg/kg. body weight of protamine sulfate was administered 10 minutes after the initial heparin injection. The clotting time, in the similarly heparinized dog, can be returned to near normal with 0.25 mg/kg. of protamine sulfate, although it did not reach pre-injection level for 30 or 40 minutes. There was no effect with the injection of 0.22 or less mg/kg. body weight of protamine sulfate. When 1.0 mg/kg. body weight of protamine sulfate was administered intravenously in normal

dogs, it protected against the subsequent injection of 0.5 mg/kg. of heparin. The extent of the protection began to diminish when heparin injection was made 30 minutes or longer after the protamine injection. No protection occurred when heparin was given 90 minutes after the protamine. The effect of 0.5 mg/kg. of heparin was also inhibited by the injection of 1.0 mg/kg. of gelatin given 10 minutes after heparin. Gelatin given prior to heparin showed some anti-heparin action. However, when larger amounts of gelatin (1.0 gm/kg.) were injected there was no immediate protection although definite protection was observed if there was a time lapse of 4 hours before injection of heparin.

Reaction of T-1824 anion and T-1824 albumin with liver slices. THOMAS H. ALLEN and PETER D. ORAHOVATS (by invitation). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York, N. Y.* The affinities of liver cells for T-1824 anion and T-1824 albumin were measured in an attempt to find the mechanisms by which this blue dye escapes from the blood strea,. Rat liver slices were rapidly prepared by freehand section, rinsed twice and suspended in 2.7 ml. of Ringer-phosphate (*pH* 7.3) of low $CaCl_2$ concentration (0.00024 M) contained in reaction vessels of Warburg respirometers. After equilibration at 37.5°C. with oxygen, 0.3 ml. of 0.0000176 M T-1824 was added from the sidebulb either as T-1824 anion or T-1824 albumin (dye in 2% bovine serum albumin). For controls 0.3 ml. of either Ringer or albumin-Ringer was used. The initial QO_2 was 9.3 and throughout was not changed by the addition of T-1824 although the slices became visibly blue in color. At various intervals after mixing with dye the slices were removed, and the suspension fluid was centrifuged for 5 minutes. The concentration of T-1824 in the fluid was determined using a König-Martens visual spectrophotometer. The maximum optical density always remained at 605 mμ for T-1824 anion and at 625 mμ for T-1824 albumin. After 200 minutes in the presence of liver slices equivalent to 10 mg. dry weight, the relative amount of added dye which leaves the suspension fluid is 36% for T-1824 anion and 18% for T-1824 albumin. Graphic analysis suggests that the dyeing process follows a second order reaction in which dye is sorbed thrice as rapidly from T-1824 anion as from T-1824 albumin.

Effect of extirpating the prefrontal suppressor area 8s on correct olfactory conditioned differential responses in dogs. WILLIAM F. ALLEN. *Dept. of Anatomy, Univ. of Oregon Medical School, Portland, Ore.* It was reported previously that prefrontal lobectomy abolished olfactory conditioned differential foreleg responses for hundreds of tests. The purpose of this study is to localize the area. The positive and negative conditioned stimuli consisted of inhalation of clove oil and asafetida. Extirpation of the cortex was accomplished by suction. It included the caudal portion of the proreus gyri and the praesylvian (orbitalis) gyri to the level of proreus sulci. In the median line the lesion extended about 5 mm. rostrad of the cruciate sulci and was about 3 or 4 mm. wide in front of the left and right praesylvian sulci. The forelegs did not show motor symptoms following the operations and there was no injury to the lateral olfactory tracts. After the extirpations, previously acquired positive conditioned reflexes from olfactory, auditory and cutaneous stimuli, required a varying number of tests before they appeared. Correct conditioned differential responses of the foreleg from olfactory stimuli were not obtainable during 4500 to 5000 tests, which were continuous over periods of 6 months. On the other hand, correct conditioned differential responses from auditory and cutaneous stimuli were always obtainable. The chief effect of the lesion was inability to with-hold foreleg flexion during a negative conditioned test or lack of correct inhibition, which occurred when area 8s was intact. A smaller ablation involving only the caudal part of the proreus gyri produced no effect on the olfactory conditioned differential responses, but like the more extensive lesion required a number of trials before the positive conditioned reflexes could be elicited.

Effect of rest intervals on maintenance of previously induced high altitude acclimatization. PAUL D. ALTLAND (introduced by HEINZ SPECHT). *Laby. of Physical Biology, Exptl. Biology and Medicine Inst., National Inst. of Health, Bethesda, Md.* This investigation was made as a part of a series of studies analyzing the mechanism of acclimatization. Maximum hematocrit values (74.0–80.0%), which have been associated with increased altitude tolerance by other investigators, were induced in Sprague-Dawley rats by 4-hour daily exposures to 18,000 and 25,000 feet simulated altitudes between ages of 14 and 100 days. Following this, cessation of exposures restored normal hematocrit values in 28–35 days. When 92 hours' rest was allowed between repeated exposures normal values were attained in 45 days. With 68 hours' rest between exposures at 18,000 feet hematocrit values leveled off at approximately 56.0% in 40 days, and at 25,000 feet they leveled off at approximately 66.0% in 20 days. With 44 hours' rest between exposures at 18,000 feet, the hematocrit values leveled off at approximately 66.0% at 20 days, and at 25,000 feet at approximately 73.0% in 20 days. When daily exposures were continued maximum levels persisted throughout 25 weeks of test. Retarded growth of male rats was observed at both altitudes (approximately 30.0% at 25,000 feet; 15.0% at 18,000 feet). Control body weight levels were

regained only in the rats of the 92-hour rest group and in those in which exposures were stopped. These experiments show that relatively high hematocrit levels may be maintained by short exposures to 18,000 or 25,000 feet at 2 or 3-day intervals, but that such acclimatization is lost completely by exposure at greater intervals.

The Rorschach test as an aid to electroencephalographic analysis. MARIE D. AMOROSO (introduced by T. C. BARNES). *Dept. of Electroencephalography, Hahnemann Medical College and Hospital of Philadelphia, Penna.* According to Darrow (*Physchol. Rev.* 54: 157, 1947) electroencephalography has contributed little to psychology. This is a result of the crude clinical electroencephalographic routine now prevalent in America where thousands of patients are rushed through the EEG lab by the neurologists. Physiological factors (blood sugar, vital capacity, skin temperature) are also completely ignored (*J. Psychol.* 22: 67, 1946; *Federation Proc.* 5: 5, 1946; *Federation Proc.* 6: 74, 1947). Personality can be related to EEG (Barnes, *Federation Proc.* 6: 75, 1947; Wheeler, *Am. Psychologist* 3: 278, 1948). In patients receiving glutamic acid Wagner (*Federation Proc.* 6: 220, 1947) found improved Rorschach responses correlated with disappearance of abnormalities in the EEG. We have found that blocking and repetition in the Roschach occurs with delta activity. Of 189 patients 25 had 'good' Rorschach, 8 had normal EEGs, 11 borderline, 6 abnormal EEGs; 81 'average' Rorschach had 34 normal, 22 borderline, 24 abnormal EEGs; and 83 'poor' Rorschach had 21 normal, 13 borderline, 49 abnormal EEGs. There is a tendency for 'poor' Rorschach scores to occur with abnormal EEGs, therefore the Rorschach serves as an aid in analysis when doubtful significance is found in the electrical picture.

Carbohydrate metabolism in the decerebrated rat. EVELYN ANDERSON and WEBB HAYMAKER (by invitation). *National Inst. of Health, Bethesda, Maryland, and Army Inst. of Pathology, Washington, D. C.* It has been demonstrated by Claude Bernard and numerous others since his time, that piqûre of the floor of the IVth ventricle or decerebration at the pontile level in rabbits and cats gives rise in less than an hour to hyperglycemia and glycosuria and that the increase in sugar is usually maintained for 3–4 hours, but may last as long as 9 hours. The decerebrated animals generally survived not more than a day or two. This communication describes the effects of decerebration on carbohydrate metabolism in rats which survived the operation 1–3 weeks. Decerebration was done at pontile and midbrain levels. Immediately following operation, blood sugar levels were determined, and a few days to 3 weeks post-operatively, glucose tolerance, insulin sensitivity, and liver and muscle glycogen studies were carried out. At autopsy the brains were fixed in 10% formalin, and the site of the lesions determined by microscopic examination. During the first few hours after pontile decerebration there was marked hyperglycemia, following which the glucose tolerance curve became markedly elevated. The animals showed a normal sensitivity to insulin, and their capacity to store fed glucose as liver and muscle glycogen alsoappeared normal.

Effect of aging on the oxygen consumption of non-nucleated erythrocytes. LUIS ANGELONE (by invitation) and CLIFFORD A. ANGERER. *Dept. of Physiology, Ohio State Univ., Columbus, Ohio.* Preparatory to further studies (Gonzalez and Angerer, 1947) on the respiratory metabolism of enucleated erythrocytes, it becomes necessary to study the effect of aging on isolated erythrocytes. These cells, obtained from 32–43 normal male white rats (100–150 gm.) by means of cardiac punctures, were pooled, washed twice, centrifuged to constant volume, resuspended in an equal volume of Krebs' solution (*p*H 7.4), and stored at 2°–8°C. Suspensions were returned to room temperature (19°–25°C.) for oxygenation and withdrawal of 2.5 ml. into each of 3–6 respirometers. Five different blood pools were studied from the first day to varying periods of time (12–22 days). Simultaneous determinations were made of bacteria, erythrocyte counts and oxygen consumption (Warburg technique). Since the general nature of all curves are identical, save for time, data from the last blood pool are presented as typical. Beginning the day after isolation the QO_2 (dry wt.) shows a progressive decrement from -0.16 mm³ by 14, 31, 44 and 44% on 2, 4, 7 and 13 days respectively; after which a 700% increase occurs at 21 days. An unexpectedly linear decrement from 4,820,000 to 1,180,000 erythrocytes/mm³ occurs at 13 days. Between 7 and 13 days a relative increase in bacteria occurs and the leveling of the curve is interpreted as due, at least in great part, to this contamination.

Dietary protein and cholic acid synthesis in dogs. J. H. ANNEGERS, and F. FRIEND (introduced by JOHN S. GRAY). *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, Ill.* This study was undertaken in order to identify the factor(s) present in protein foods which is responsible for their stimulatory effect on cholic acid output. Nine cholecystonephrostomized dogs were used; bile-urine mixtures were analyzed spectrophotometrically for cholic acid (Irvin, Johnston, and Kopola, *J. Biol. Chem.*, 153: 439, 1944). The animals were given a basal daily diet of one half can of 'Pard' alone, then 25 and 50 gm. daily of crude casein, vitamin-free casein, casein hydrolysate ('Amigen'), zein, or gelatin were added. Each diet was fed for 7 days and cholic acid output determined the last 5 days. Cholic acid output in-

creased in linear fashion with the quantity of casein or zein added to the basal diet. Vitamin-free casein increased cholic acid output to a slight but significant extent. Neither casein hydrolysate nor gelatin significantly altered cholic acid excretion when added to the basal diet. These findings suggest that some factor (s) other than amino acids is necessary for the increase in cholic acid synthesis which follows feeding of protein foods.

Mechanism of the biosynthesis of propionic acid. D. S. ANTHONY, S. F. CARSON, M. KUNA, and E. F. PHARES (introduced by ALEXANDER HOLLAENDER). *Biology Division, Oak Ridge National Laby., Oak Ridge, Tenn.* Experiments on the biosynthesis of propionic acid (by *Propionibacterium pentosaceum*) from 3-carbon substrates have indicated that C_2 and C_4 compounds are intermediate. The following C^{14} labeled compounds were used as tracers: $C^{14}H_3 \cdot COOH$, $CH_3 \cdot C^{14}OOH$, $CH_3 \cdot C^{14}O \cdot COOH$ and $C^{14}OOH \cdot CH_2 \cdot CH_2 \cdot C^{14}OOH$. Three pathways from a 3-carbon substrate to propionate were indicated, namely, *1*) direct conversion without breakdown of the carbon skeleton, *2*) conversion through acetate and succinate, and *3*) conversion through oxalacetate and succinate. Since this is an anaerobic process, the amount of propionate formed from each of the several mechanisms depends to a large extent upon the redox state of the substrate. Determination of the extent of each of the several mechanisms was calculated from the relative specific activities of the individual carbon fragments after separation and isotopic degradation of the end-products.

Effect of vagotomy on gastric secretion and emptying time in the dog. F. ANTIA (by invitation) and A. C. IVY. *Dept. of Clinical Science, Univ. of Illinois, College of Medicine, Chicago, Ill.* Secretory studies were made on 8 dogs with gastric fistulas before and after vagotomy. After collecting a 30-minute basal sample 0.1 mg/kg. histamine dihydrochloride was injected subcutaneously and samples were collected every 30 minutes for 2 hours. About 10 experiments were made before vagotomy and 10 after vagotomy in each dog. The average HCl output in response to histamine after vagotomy expressed as percentage of the average prevagotomy output was: 103, 77, 69, 53, 51, 47, 33, and 26 with an average of 57%.

Six of the same dogs were studied in the same fashion before and after vagotomy using 1 mg. of urecholine as the secretory stimulus. The response to urecholine varied considerably both before and after vagotomy. After vagotomy the average response to urecholine expressed as a percentage of the average prevagotomy output was: 140, 105, 36, 36, 34 and 12 with an average of 61%. After vagotomy no gastric secretory response occurred in any of the dogs when insulin was given intravenously (1.2 mg/kg.). The response to dimethylallyl-5-ethyl-barbituric acid was also abolished by vagotomy. The time required for 80% emptying of a test meal of 250 cc. of boiled milk or 200 cc. boiled milk plus 50 cc. of corn oil was studied in 4 dogs. The gastric emptying time for either meal was not significantly altered by vagotomy, thus the inhibitory effect of fat was not changed by vagotomy.

Oxygen consumption of the descending aortae of adrenalectomized rats in presence of various substrates. PHYLLIS M. ARSCOTT (by invitation) and CLIFFORD A. ANGERER. *Dept. of Physiology, Ohio State Univ., Columbus, Ohio.* Young, male, white rats, weighing between 75 and 135 gm., were used in the following experiments which treat of isolated aortae and urinary bladders. The O_2 consumption (QO_2) was studied by the Fenn technique. When aortae from adrenalectomized rats were compared with those from normal rats, there was found a decrease of 28% in the QO_2 values for the former group of rats. This figure is statistically significant. There was no observable change under the same conditions for the QO_2 values for urinary bladders of the same groups of rats. The addition, respectively, of various substrates to the aortae of normal rats gave the following percentage differences in QO_2 when compared to the aortae of normal rats without substrate: glucose −9%, lactate +18%, pyruvate −21%, succinate +214%; while under the same conditions but for aortae of the adrenalectomized rats the percentage differences were as follows: glucose −11%, lactate +126%, pyruvate +19%, and succinate +171%. The net changes between the respective pairs of results were: glucose +2%, lactate +108%, pyruvate +40%, and succinate −43%. Since the urinary bladder is composed of connective and smooth muscle tissue which simulates the histology to a certain extent of the tunicae externa and media, the change of respiratory metabolism of the aorta following adrenalectomy is related to the endothelium (tunica intima) and yellow elastic tissue, or both. If this is true, then it appears that metabolism and certain well-known permeability changes of the capillary may be correlated.

Reflex respiratory and circulatory action of veratridine on cardiac, pulmonary and carotid receptors. DOMINGO M. AVIADO, JR. and ROBERT G. PONTIUS (introduced by CARL F. SCHMIDT). *Laby. of Pharmacology, Univ. of Pennsylvania, Philadelphia, Penna.* Intravenous injection of the veratrum alkaloids has been repeatedly shown to cause reflex apnea, bradycardia and hypotension by acting on receptors in the chest. Almost all previous attempts at localization of the receptors were on open-chest preparations and the observations were on the circulatory reflexes. Catheterization of the different parts of the cardio-pulmonary circulation of anesthetized dogs (morphine and chlorolose) has enabled the localization of the receptors responsible

B & Z Library

for the reflex apnea. Injection of veratridine in doses of 1 microgni/kg. into the left or right pulmonary artery or into the artery far out in the substance of the lungs was followed by an immediate apnea, and a hypotension and bradycardia about 5 seconds later. The apnea is due to the drug action on pulmonary receptors because cold block of the ipsilateral cervical vago-sympathetic trunk eliminated the reflex apnea without noticeably changing the circulatory response. Subsequent warming was followed by the return of the apneic response to the drug. The cardiac receptors were stimulated as long as a sufficient concentration of the drug could reach the coronary artery. Although their response was predominantly circulatory, they slightly inhibit respiration. There are receptors in the carotid region, exclusive of the carotid sinus pressoreceptors, that respond to veratridine with hyperpnea, bradycardia and hypotension. The chemoreceptors sensitive to cyanide can explain the hyperpnea but not the circulatory effects.

Hyperplasia of chromaffin material in the sympathetic paraganglia of adrenalectomized rats. H. BACCHUS (by invitation), A. DURY, and D. B. YOUNG (by invitation). *Depts. of Physiology, and Zoology, George Washington Univ., Washington, D. C.* Histological sections of paraganglia of the coealic and inferior cervical plexi of bilaterally adrenalectomized and sham adrenalectomized rats 3, 4, 5, 6, 7 and 9 weeks post-operatively were examined for possible alteration and compensatory changes in their chromaffin content. An intense chromaffin reaction and hyperplasia was found in the paraganglia of the adrenalectomized rats. This was not observed in the paraganglia of the sham-adrenalectomized or normal controls. The chromaffin material was diffusely distributed and showed evidence of hyperplasia and hypertrophy in the paraganglia removed from rats which were 3 and 4 weeks post-adrenalectomized. The hyperplasia assumed a concentrated and nodular character in the paraganglia of rats which were 5 through 9 weeks post-adrenalectomy. These nodular areas in the paraganglia resembled adrenal medullary tissue. Such changes were not observed in the sham-adrenalectomized rats. This alteration in the chromaffin content of the paraganglia of the coeliac and cervical plexi suggest a compensatory reaction which might also be a site for the elaboration of adrenalin in adrenalectomized rats.

The effect of the bulbar facilitatory and inhibitory systems on spinal reflex activity. L. M. N. BACH. *Dept. of Physiology, School of Medicine, Tulane Univ., New Orleans.* Because of the extreme importance of the bulbar reticular formation in facilitation and inhibition of both reflexly and cortically induced movement, it seemed of interest to determine the effect of this system on various facets of spinal reflex activity. Animals were decerebrated under ether by either the ischemic method or by a new discrete method to be described elsewhere. Kymograph recordings were made of the reflex contractions of the tibialis anticus and the gastrocnemius muscles in response to stimulation of the ipsilateral plantar and popliteal nerves and to the contralateral popliteal nerve. Coincident stimulation of the bulbar inhibitory system (which alone had no obvious effect) caused a diminution in the degree of both reflex contraction of the tibialis muscle and reflex inhibition of the gastrocnemius muscle. The amount of rebound inhibition and contraction respectively in these two muscles was considerably increased. Bulbar facilitation causes an increase in the reciprocal innervation phenomenon by enhancing the contraction of the tibialis and the inhibition of the gastrocnemius following stimulation of the contralateral popliteal nerve. Reflex after-discharge is increased also as a result of coincident bulbar facilitation. The unexpected effects of the bulbar systems on reflex inhibition may indicate that two different processes of inhibition may obtain at the motoneuron. Studies are being carried out on the effects of these systems on occlusion and fractionation and their significance in the production of spinal shock.

The effect of local cooling and heating of the finger and wrist during exposure to high ambient temperature. MORTIMER E. BADER and JERE MEAD (introduced by H. S. BELDING). *The Quartermaster Corps, Climatic Research Laby., Lawrence, Massachusetts.* Utilizing plethysmograph cups containing salt solution bath, surrounded by a thermoregulated air stream, with thermocouples on the skin beneath the cups as well as in the bath, measurements of blood flow through finger and wrist (a 1″ segment approximately 1″ above the radial prominence) were made in a warm environment (32°C.). Control observations were made on one arm while local cooling and heating of the two regions were carried out on the other arm.

With a bath temperature maintained at 0°C. the finger skin temperature fell to and remained approximately at 7°C., but blood flow through the cooled finger did not differ significantly from base line values prior to cooling, or from the control values in the uncooled finger. Stimuli such as pain, startle and deep respiration produced sudden diminution in blood flow through both fingers; the decrease was transient, blood flow rising rapidly to normal levels within 2 minutes. The cooled finger, however, responded with much greater diminution than did the control. Local warming (bath 44°C. skin 43°C.), of the finger produced no significant increase in blood flow. In the wrist, local cooling (bath 0°C. skin 12°C.) did produce a decrease in blood flow; warming increased the flow above base line levels.

In an individual exposed to 32°C., local thermal stimuli do not appreciably affect finger blood flow, except that cooling increases the sensitivity to some general vasoconstrictive stimuli. In the wrist, the flow is directly responsive to local temperature changes. The presence of arteriovenous shunts in the fingers may be a factor in this difference.

Renal and hormonal mechanisms of cold diuresis. RICHARD A. BADER, JOHAN W. ELIOT, and DAVID E. BASS (introduced by H. S. BELDING). *The Quartermaster Corps, Climatic Research Laby., Lawrence, Massachusetts.* The mechanisms of cold diuresis have been studied by exposing men at 15°C., reclining nude except for shorts, and comparing with results of exposure at 24°–27°C., covered by blankets. Endogenous "creatinine" clearances in 9 experiments showed no significant change in glomerular filtration, and in 16 experiments there was no significant change in either endogenous "creatinine" or para-aminohippuric acid clearance, during cold diuresis. In 12 experiments there was a substantial reduction in cold diuresis during standing, as compared with reclining. In 12 experiments cold diuresis was decidedly inhibited following 15 minutes of walking.

During cold diuresis the specific gravity and chloride concentration of the urine fell as the volume rose, and each change appeared to be correlated with the previous state of hydration and chloride content of the diet. Use of an index of chloride reabsorption showed that chloride tended to be reabsorbed to a greater degree than water.

In 27 experiments three men received small intramuscular doses of either .015, .03, .06 or .12 units per kg. of pitressin at the beginning of each exposure, either cold or warm. Inhibition of cold diuresis was proportionately longer lasting following the larger doses, but even the smallest dose reduced urine flow for 1½ hours to the same small output seen in the warm exposures, with or without pitressin. The strong similarity shown between water and cold diuresis and the extreme sensitivity to pitressin constitute suggestive evidence that cold diuresis is controlled by the posterior pituitary gland.

Hepato-renal factors in circulatory homeostasis. XXIII. The antidiuretic action of crystalline VDM (ferritin, apoferritin). SILVIO BAEZ (by invitation), ABRAHAM MAZUR and EPHRAIM SHORR. *Dept. of Medicine, Cornell Univ. Medical College, and The New York Hospital, New York City.* A previous study from this laboratory established the antidiuretic action in animals (dogs, rabbits) of partially purified hepatic VDM concentrates. The subsequent identification of hepatic VDM, as ferritin (Mazur and Shorr, *J. Biol. Chem.*, 176, 771, 1948) has made it possible to determine whether the antidiuretic effect was due to VDM or to other unidentified components of the crude concentrates. Crystalline ferritin was prepared from horse spleen and dog liver. Experiments were carried out on rabbits and dogs. A priming dose of ferritin (500–600 gamma ferritin nitrogen) was given intravenously 5 minutes before oral hydration with 35–40 cc/kg. of water. Then, a total of 200–300 gamma/kg. of ferritin nitrogen was infused intravenously in 50 cc. of saline over 30 minutes. In control experiments, the peak of diuresis was reached in 60–90 minutes and the ingested water excreted in 180–270 minutes. With ferritin, the peak of diuresis occurred at 170–270 minutes; excretion was complete in 300–360 minutes. Doses of 50–180 gamma ferritin nitrogen were ineffective. Antidiuresis was also observed in dogs given dog liver ferritin; this excluded the possibility of non-specific effects of heterologous protein. Crystalline horse spleen apoferritin, which has identical vasodepressor effects with ferritin, exerts comparable antidiuretic action. Rabbits immunized against horse spleen ferritin failed to exhibit antidiuresis following intravenous horse spleen ferritin while antibody to ferritin was detectable in blood. The relation of the posterior pituitary to the antidiuretic action of ferritin and apoferritin will be reported.

Urinary excretion of calcium during intravenous infusion. STANLEY M. BALL (by invitation) and A. V. WOLF. *Dept. of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.* Female dogs received continuous intravenous infusions of calcium chloride or gluconate solution (concentrations ranging from 0 to more than 20 mEq Ca per liter) at ca. 4 cc/min for 5 hours. The diuretic effects of these solutions were not significantly different from those of 5½% glucose solution (water). The plasma Ca level is not effectively regulated by renal activity alone, there being relative retention of Ca to water even when solutions less concentrated than plasma Ca were administered. Thus Ca has no clear threshold of retention and its limiting isorrheic concentration under these conditions is smaller than the normal plasma concentration of Ca. No prominent specific effects of intravenous Ca were observed on the urinary excretion of sulfate, phosphate, bicarbonate, potassium, magnesium, hydrogen ion, sodium, or chloride, although some increased urinary excretion of the latter two have been observed with toxic doses of Ca. Loads of Ca in excess of about 24 mEq in a 15 kg. dog, created within 5 hours of steady infusion, lead to vomiting. The velocity constant of excretion for Ca (minute rate of excretion per unit load) is of the order of 1×10^{-4} to 1×10^{-3} min^{-1}, somewhat less than previously found values for chloride from sodium chloride.

Comparative evaluation of several methods for determining alveolar gas tensions in man. EARL S. BARKER (by invitation), ROBERT G. PONTIUS (by invitation), DOMINGO M. AVIADO, JR. (by invitation) and CHRISTIAN J. LAMBERTSEN. *Laby. of*

Pharmacology, Univ. of Pennsylvania, Philadelphia, Penna. Simultaneous measurements of alveolar gas tensions by 4 methods and of arterial blood gas tensions by one method were made in 11 human subjects in an attempt to establish comparative values and to determine the possible existence of a direct alveolar gas sampling method which could, in the absence of a direct standard of reference, be used to provide values comparable to those obtained by indirect calculation of mean alveolar gas tensions. Blood tensions were determined by a method having a standard deviation of ±2.0 mm. Hg. Alveolar tensions were determined by the end-expiratory Haldane-Priestley technique, the automatic end-normal expiratory method, indirect calculation of effective gas tensions and sampling of the last part of each normal expiration from the tracheal bifurcation using a catheter inserted through the cricothyroid membrane.

	pCO_2	pO_2
Arterial blood	40.5	96.6
Alveolar gas		
Haldane-Priestley End-Expiratory	40.7	97.9
End-normal Expiratory (tracheal)	40.5	99.7
'Effective'	40.5	100.3
End-normal Expiratory (beyond mouth)	38.6	103.1

Statistical analysis revealed no significant differences among *a*) pCO_2 of blood, Haldane-Priestley and tracheal samples, *b*) pO_2 of blood and Haldane-Priestley samples, *c*) the alveolar pO_2 obtained by the 'effective', the Haldane-Priestley and the tracheal methods. All other relationships showed statistically significant differences.

Evaluation of potential thyroxine inhibitors. S. B. Barker and (by invitation) C. E. Kiely, Jr., S. Wawzonek and S. C. Wang. *Depts. of Physiology and Chemistry, State Univ. of Iowa, Iowa City, Iowa.* It is desirable to have procedures available for determining whether various chemical compounds are capable of interfering with the peripheral action of thyroxine in the mammalian organism. Because of the interest of this laboratory in energy metabolism, one test has centered around O_2—consumption studies on groups of trained albino rats. Such a method enables the measurement of the effect of appropriate dosages of potential thyroxine antagonist on endogenous as well as exogenous thyroxine. Another technique takes advantage of the increased sensitivity of the animal without thyroid to small doses of estrogen. This heightened sensitivity can be depressed by injection of thyroxine. Presumably, any inhibitor of thyroxine action should restore the greater sensitivity to estrogen. Since the vaginal smear changes can be followed without damage to the animals, sacrifice is unnecessary, and the same operated rats can be used for several tests, with an adequate interval of rest. Several compounds structurally related to thyroxine have been tried, among which are the following: 3,5-dibromotyrosine, 3,5-diiodotyrosine, 3,5-diiodo-4-hydroxybenzoic acid, 4-(p-methoxyphenoxy)-3,5-dibromocinnamic acid, 4-(p-methoxyphenoxy)-3,5-diiodocinnamic acid, and (furnished by Dr. M. J. Schiffrin of Hoffmann LaRoche, Inc.) N-(4-hydroxy-3,5-diiodobenzoyl)-3- (4-hydroxy-3,5-diiodophenyl)-alanine. None of these has been found by either test to show significant evidence of antagonistic effect. Some exhibit a weak thyroxine-like activity.

Quantitative method of estimating the physiological effects of deep-breathing on the electroencephalogram. T. C. Barnes and Marie D. Amoroso. *Dept. of Electroencephalography, Hahnemann Medical College and Hospital of Philadelphia, Penna.* Methods of scoring the electroencephalogram in acapnia (*J. Psychol.* 22: 67, 1946; *Federation Proc.* 6: 74, 1947; *J. Gerontology* 3 Supplement to No. 4, p. 4, 1948) have a greater diagnostic value than crude clinical electroencephalographic procedure. Scores for abnormal waves range from −15 (most abnormal) to −2 (normal). In 276 patients these were: 15 to 19 years −7.7, 20 to 24 −5.5, 25 to 29 −4.2, 30 to 34 −5.0, 35 to 39 −3.5, 40 to 44 −2.0, 45 to 49 −2.8, 50 to 54 +0.12, 55 and over −1.7. When age and other factors are scored the index is as follows: 15 to 19 +3.0, 20 to 24 +2.2, 25 to 29 +1.9, 30 to 34 −1.4, 35 to 39 −0.76, 40 to 44 −0.77, 45 to 49 −0.33, 50 to 54 −2.0, over 55 −1.6. It is concluded that age together with physiological factors play an important part in the effect of deep breathing on the electroencephalogram and should be taken into consideration when a final analysis is made.

CO_2 pressure gradient across the placenta. Donald H. Barron. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* The carbon dioxide pressure gradient between the fetal and maternal sheep blood separated by the syndesmochorial (5 layer) placenta has been estimated in animals in the last third of the gestation period. The gas content of samples drawn (with the usual precautions and with animal under a spinal anesthesia) from the uterine artery, uterine vein, umbilical artery, umbilical vein, have been referred to CO_2 dissociation curves for the estimation of pressures. The results indicate that the CO_2 pressure in the uterine artery varies between 12 and 18 mm. Hg whereas the pressure in the umbilical vein fluctuates between 32 and 40 mm. Hg; the average pressure difference between the fetal and maternal blood appears to be about 20 mm. Hg. Further, the *p*H of the fetal blood leaving the placenta appears to be fairly constant at 7.35–7.42, and points to the possibility that the fetal circulation through the placenta may be adjusted to regulate the *p*H of the blood reaching the fetal brain.

Limitations of the polarographic method of determining the oxygen tension in whole blood. J.

PERCY BAUMBERGER, GABOR MARKUS (by invitation) and KATHLEEN BARDWELL (by invitation). *Physiology Dept., Stanford Univ. School of Medicine, Stanford, Calif.* In 1938 Baumberger measured the O_2 tension of dilute blood polarographically in order to determine the oxygen dissociation curve of oxyhemoglobin. In 1942 Berggren (*Acta Physiol. Scand.*) determined the O_2 tension of arterial blood using separated plasma for his polarographic determinations because the presence of hemoglobin interfered with the measurements. In 1947 Baumberger (*Abst. Int. Physiol. Cong., Oxford*) reported that the O_2 tension of whole blood could be determined polarographically if cyanide were added. Further work, however, has shown that cyanide leads to correct results only under very limited conditions. Whole blood gives approximately the same polarographically determinable O_2 tension as Ringer's solution exposed to the same gas, provided the hemoglobin is fully saturated. The O_2 tension must, however, be measured at an applied voltage of -1.5 vs. sta. cal. elect. If measured at -0.5 Volt, cyanide must be added in low concentration to inhibit the catalytic decomposition of the electrolytically formed hydrogen peroxide. As the ratio of hemoglobin/oxyhemoglobin increases, the contribution of oxygen dissociated from oxyhemoglobin causes a difference to develop between blood and Ringer's (confirming Berggren). Factors such as *p*H and temperature that displace the oxygen dissociation curve affect the size of this difference and it would appear that a useful quantitative relationship can be worked out. When blood is sufficiently diluted (1:100) this discrepancy is not apparent as demonstrated by the correct oxygen dissociation curves for oxyhemoglobin obtained by a combination of spectrophotometric and polarographic determinations.

Intestinal motility and blood flow as influenced by arterial pressure. JOHN W. BEAN and M. SIDKY MOHAMED (by invitation). *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* Isolated loops of dog intestine were perfused with heparinized dog blood. Sudden elevation of constant arterial pressure induced tonic contraction and augmented rhythmic contraction; lowered pressure also frequently increased tonic and rhythmic contractions for short periods initially, later decreased them. Occlusion of venous outflow while maintaining arterial pressure constant, immediately augmented tonus, rhythmic contractions, and venous pressure which was further increased by rhythmic contractions. Opening outflow after brief occlusion resulted in a temporary rapid flow above pre-occlusion rate but tonus and motility decreased. Arterial occlusion caused an immediate gradual drop in arterial pressure, a gradually diminished flow to zero, a decrease motility and tonus frequently after an initial increase. Arterial deocclusion immediately increased the flowfar in excess of either the preocclusion value or that following deocclusion of the vein, suggesting vascular dilatation during lowered arterial pressure. Simultaneous venous and arterial occlusion decreased arterial pressure, raised venous pressure temporarily, slightly increased motility initially then decreased it. Sudden elevation of arterial pressure by deoccluding the artery (vein still occluded) sharply increased motility and tonus, and venous pressure increased rhythmically. The records indicate, that changes in arterial and venous pressure *per se* influence intestinal motility and tonus appreciably; they also show that changing a constant arterial pressure to a slow pulsatile pressure alters gut motility by means other than blood flow changes. Shifting from constant to pulsatile pressure caused a reversible decrease in blood flow indicative of vasoconstriction.

Observations on congenital heart cases with the oxyhemograph. VIVIAN G. BEHRMANN, FRANK W. HARTMAN (by invitation), ROBERT F. ZIEGLER (by invitation) and CONRAD R. LAM (by invitation). *Depts. of Labys., Cardiology and Surgery, Henry Ford Hospital, Detroit, Mich.* Oxyhemography was employed during operations for tetralogy of Fallot (16 patients), patent ductus arteriosus (2 patients) and coarctation of the aorta (2 patients). Every tracing included the pre-anesthesia period, the induction and fluctuations occurring throughout the surgical procedure. A calibration for each O_2 saturation curve was obtained through Van Slyke analyses on at least two arterial blood samples. The effect of anesthetic agents, drugs and intravenous solutions administered during the operation on the arterial O_2 level are shown and discussed. The usefulness of the continuous blood O_2 curve as an index of the establishment of a more adequate pulmonary circulation is well illustrated. In 8 instances, preoperative and postoperative oxyhemograph studies, which included a determination of the O_2 saturation time and the effect of exercise, have been helpful in the evaluation of the congenital heart abnormalities as well as the benefit derived from surgery. These data indicate that continuous blood O_2 saturation recordings should prove valuable not only to the cardiologist but also to the surgeon and anesthetist in the treatment of congenital heart disease.

Digital skin temperature and blood flow relationship following change in environmental temperature. HARWOOD S. BELDING, JERE MEAD (by invitation) and MORTIMER E. BADER (by invitation. *The Quartermaster Corps, Climatic Research Laby., Lawrence, Massachusetts.* Digital skin temperatures and blood flows in the terminal phalanx (venous occlusion plethysmography) were recorded on men wearing shorts and exposed for three hours in a room at 32°C., shifted abruptly to 13° ambient temperature and later returned to the 32°C. tem-

perature. The subjects lay supine and were wheeled between the constant temperature rooms to obviate the effects of exercise.

In the cold, blood flows decreased immediately and precipitously and usually reached values of 3% or less of the initial levels within three minutes. Digital skin temperatures fell relatively less rapidly, and closely paralleled temperatures in digits in which arterial inflow was obstructed. In the warm room, after cold exposures varying from 30 minutes to 2 hours, digital temperatures rose precipitously and initially paralleled temperature rises in arterially occluded digits. Return of blood flow was strikingly less rapid; e.g., in the first 90 minutes at 32°C. following a 2 hour cold exposure, digital temperatures returned to within 20% of the control level, but blood flow was still 90% below the control level. Not until 40 minutes later did the flow increase to control levels.

Conclusions as to the state of digital blood flow derived from digital skin temperature alone, in other than constant ambient conditions, are limited by the fact that a change in ambient temperature may directly produce marked digital skin temperature changes independent of digital blood flow, and hence, of body heat economy.

Body water compartments in man. EUGENE Y. BERGER (by invitation), MARCELLE F. DUNNING (by invitation), BERNARD B. BRODIE (by invitation), and J. MURRAY STEELE. *Depts. of Medicine and Biochemistry, New York Univ. College of Medicine, and Research Service, Third New York Univ. Medical Division, Goldwater Memorial Hospital, New York, N. Y.* Recent measurements of body water by the dilution of deuterium oxide and of antipyrine have shown that the average percentage of water is formales about 53%, which figure is considerably lower than the generally accepted average of 70%. Further, the proportion of water exhibits considerable variability. The variability may be explained on a basis of fat content since measurements of specific gravity of the body have shown that fat and water content bear an inverse relationship. When the extracellular fluid space as measured by bromide was subtracted from total body water space, the intracellular fluid space obtained by difference was obviously too small to be reasonable. This suggested that neither bromide nor chloride was confined to extracellular water. Hence, a substance whose volume of distribution was smaller than that of bromide was likely to be a closer measure of extracellular fluid. Inulin was selected because it is known not to enter cells, yet passes the glomerular membrane and enters the pleural and peritoneal spaces. The inulin and bromide spaces in nephrectomized dogs and in patients with poor kidney function were compared. Inulin space was about half that of the bromide space. Antipyrine space and inulin space were also measured simultaneously in normal human subjects, using an infusion technique previously described (*Proc. Soc. for Exptl. Biol. & Med.*, 68: 507, 1948). The results indicate that extracellular space constitutes about 15% and intracellular space about 40% of the body weight in normal subjects.

Mechanisms of potassium excretion in the normal dog. ROBERT W. BERLINER, JAMES G. HILTON, and THOMAS J. KENNEDY, JR. (introduced by JAMES A. SHANNON). *Research Service, First (Columbia Univ.) Division, Goldwater Memorial Hospital, and Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., New York, N. Y.* Certain observed reciprocal relationships between sodium and potassium excretion suggest an exchange of K for Na ions, either directly or by secretion of K and reabsorption of Na with some common anion. The existence of such an exchange can be demonstrated by obtaining in the urine larger amounts of secreted K than of anions which might have been secreted with it. These conditions have been fulfilled in experiments where the chief urinary anion was ferro-cyanide the minimum secreted (excreted minus filtered) K exceeding, by considerable margins, the sum of chloride, bicarbonate, sulfate and phosphate. Similar conclusions to be drawn from experiments in which thiosulfate was the major anion, require the additional assumption that sulfate is not secreted. In experiments where Na_2HPO_4, $Na_2S_2O_3$ or $Na_4Fe(CN)_6$ is infused and K substituted for Na in the infusion, the initial effect is a marked increase in the excretion of K accompanied by a sharp rise in urinary bicarbonate and chloride excretion. The increases occur while excreted K is well below that filtered. Later *p*H and Cl excretion level off or drop while K excretion continues to rise, with Na excretion now dropping. These phenomena suggest that the initial increase in K excretion is due to decreased tubular reabsorption rather than secretion.

Alcohol and sucrose in the production of liver damage in rats. C. H. BEST, W. STANLEY HARTROFT (by invitation), C. C. LUCAS and JESSIE H. RIDOUT (by invitation). *Banting and Best Dept. of Medical Research, Univ. of Toronto, Toronto, Canada.* Although other investigators have studied the influence of alcohol upon the production of cirrhosis in several species of animals, no one, as far as we are aware, has examined the effect of isocaloric paired-feeding, i.e., supplying the non-alcoholic control animals with only the same quantity of basal diet as was eaten by the animals consuming alcohol (15% solution replacing drinking water) and equalizing the caloric intake by adding an appropriate amount of carbohydrate (powdered sucrose) to the food of the controls. About 200 white rats of the Wistar strain, initially weighing 150 grams, divided into 12 groups, were studied under these experimental conditions for 6 months, some being given lipotropic

supplements (choline, methionine or protein). Liver damage frequently attributed to alcohol appeared when sugar was substituted for it isocalorically. The inclusion of 0.05% of choline chloride in the basal diet (casein 10, gelatin 5, zein 3, cystine 0.3, fat 12, celluflour 2 and carbohydrate, with adequate minerals and vitamins, including α-tocopherol) sufficed to prevent the appearance of more than slight fatty change in the livers of the majority of animals on the basal ration (total lipids under 15%). An adequate supply of choline, or precursors, in relation to the total caloric intake (including alcohol or sugar supplements) prevented liver damage. Addition of sucrose to the basal diet produced as severe liver damage as did an isocaloric intake of alcohol, even to the point of fibrous tissue proliferation observed in a few rats. This type of experiment is, at present, being carried out in very young rats and in older rats receiving diets in which the available lipotropic factors have been more drastically reduced.

A phase-boundary mechanism for impulse formation, transmission and synaptic properties of nerve. R. BEUTNER and T. C. BARNES. *Dept of Pharmacology, Hahnemann Medical College and Hospital of Philadelphia, Penna.* Previous reports (*Science* 94: 211, 1941) described the negative phase-boundary electrical potential of acetylcholine at the interface between oil and saline forming a wave like the nerve impulse (*Internat. Physiol. Congress* 390, 1947; *Federation Proc.* 6: 73, 1947). Our present experiments demonstrate the transmission of this wave. A horizontal glass tube 60 × 1.2 cm. (paraffined to reduce conductivity over the glass surface) contains a continuous layer of guaiacol and resin mixture with a supernatant saline layer. At each end of the horizontal tube there is a U-tube—one for introduction of the acetylcholine and one for the recording electrodes on each side of the oil layer. The addition of 0.1 cc. of saline containing 0.1 mg. acetylcholine in the U-tube at one end produces a negative wave of 3 mv. lasting 0.15 sec. recorded from the other U-tube at the opposite end of the horizontal tube. Multichannel electroencephalographic recording showed that the velocity is over 1 m. per sec. Conduction may be direct as between two nodes of Ranvier (Stampfli, *Internat. Physiological Congress* 218, 1947). The sensitivity of the synapse to acetylcholine was duplicated by adding more resin to the middle horizontal tube and filling the terminal U-tubes with oil only. In this modified elongated oil-cell acetylcholine produced a transmitted wave when added to the ends or synapses but had no effect when added to the middle of the central tube or axon.

Cortical response to light in the unanesthetized rabbit. REGINALD G. BICKFORD (introduced by CHARLES F. CODE). *Section on Physiology, Mayo Foundation and Mayo Clinic, Rochester, Minn.* The basic response of the cortex to a light flash of short duration in the unanesthetized rabbit consists of an initial fast diphasic deflection (*a*-wave) at 40 milliseconds, followed by a large slow and variable diphasic deflection (*b*-wave), swinging negative and then sharply positive, and delayed at its negative peak by 200 milliseconds from the flash. The *'a'*-wave, enhanced in size and changed to a predominantly surface positive deflection by barbiturate narcosis, has been studied by previous workers. Because of its variability and the fact that it is suppressed by barbiturate narcosis, the *'b'*-wave has received little attention. Investigation has shown that the intermittency of the *'b'*-wave in the unanesthetized rabbit results from the disturbing effect of sensory stimuli. Thus, the *'b'*-wave may be promptly suppressed (without affecting the *'a'*-wave) by adequate stimulation of the visual, auditory, olfactory or tactile systems. The suppression cannot be maintained indefinitely by continued stimulation of one system. Thus, stimuli appear to be effective in proportion to their 'surprise value.' Weak sensory stimuli may cause suppression in a small part of the responding striate area. It seems probable that the *'b'*-wave represents the synchronized response of neurons concerned in elaboration of visual material, receipt of which is signaled by the *'a'*-wave. Arousal stimuli cause a temporary desynchronization of the former mechanism. This phenomenon is of particular interest, since a similar suppression of the responses to light stimulation can be demonstrated in electro-encephalograms of human beings.

Cardiac oxygen consumption and efficiency in man. R. J. BING, and (by invitation), M. HAMMOND, J. HANDELSMAN, S. POWERS, AND F. SPENCER. *Dept. of Surgery, Johns Hopkins Univ. School of Medicine, and Johns Hopkins Hospital, Baltimore, Md.* The O_2 consumption and efficiency of human heart muscle were obtained *in vivo*, in patients with anemia, essential hypertension, coarctation of the aorta, and aortic stenosis and insufficiency. Several patients were in cardiac failure resulting from mitral stenosis and insufficiency. The coronary blood flow was determined with the nitrous oxide method and coronary vein blood was collected through a catheter inserted into the coronary sinus. The O_2 consumed per 100 gm. of left ventricular muscle was obtained from the coronary blood flow per 100 gm. of cardiac tissue and the difference in O_2 content between coronary arterial and venous blood. Left ventricular weight was obtained from the table of Smith, and the left ventricular efficiency was calculated from the work and the energy cost of the left ventricle. Since figures for heart weight cannot be obtained accurately, values for the efficiency are approximations.

In normal man the coronary flow averaged 65 cc/min/100 gm. cardiac tissue, the arteriovenous

oxygen difference 11 vol. %, and the O_2 consumption 7.8 cc/100 gm/min. In anemia the coronary flow was increased, the arteriovenous O_2 difference and the O_2 consumption were reduced. In essential hypertension the coronary flow was normal, the arteriovenous O_2 difference and the O_2 consumption were decreased. In coarctation the coronary flow, the arteriovenous O_2 difference, and the O_2 consumption were markedly increased. In cardiac failure the coronary flow was reduced but the arteriovenous O_2 difference was increased. The O_2 consumption was slightly above normal. As a result of these changes the cardiac efficiency was highest in essential hypertension and lowest in cardiac failure. The lowered cardiac efficiency in failure was primarily the result of decreased left ventricular work. Correlation between the work and the O_2 consumption of the left ventricle was good except in essential hypertension and cardiac failure.

Properties of the antidiuretic substance in the blood of normal and adrenalectomized rats. J. H. BIRNIE (by invitation), W. J. EVERSOLE (by invitation), W. R. BOSS (by invitation), C. M. OSBORN (by invitation) and ROBERT GAUNT. *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.* It has been reported (Birnie, Jenkins, Eversole and Gaunt—in press) that the blood of normal rats contains an antidiuretic, chloruretic substance (ADS). It was readily detectable by a modified Heller and Urban assay procedure in 1 ml. quantities of fresh serum injected intraperitoneally. The serum lost its activity upon standing at 9°C. for 12 hours, and was present in greater quantities in adrenalectomized than in normal rats. The latter observation will presumably help explain the well-known failure of water diuresis in adrenalectomized animals. Newer experiments have shown the following: *1)* ADS was not detected in 1 ml. samples of the serum of rats hypophysectomized from 4 to 11 days. *2)* The ADS of normal rat serum (1 ml.) does not affect renal glomerular filtration as indicated by creatinine clearance tests and presumably therefore works by stimulating the tubular reabsorption of water. *3)* The ADS is active when injected subcutaneously but less effective than by intraperitoneal administration. *4)* Unlike the ADS, an isotonic gelatin solution, prepared for blood substitute use, has no effect on diuresis in 1 ml. doses. *5)* Crude protein solutions, injected intraperitoneally, may inhibit diuresis by other mechanisms; they differ from ADS in that they act less promptly and are not chloruretic. All of these observations are consistent with the idea that the circulating ADS is the antidiuretic hormone of the posterior pituitary.

Analysis of factors affecting the assay of gastric secretory depressants. D. BLICKENSTAFF (by invitation) AND M. I. GROSSMAN. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* In order to design an efficient and accurate assay procedure, the factors which contribute to the variability of results in assaying gastric secretory depressants were studied statistically. The day to day and hour to hour variability in the output of HCl in response to small doses of histamine administered subcutaneously every 10 minutes was determined in Heidenhain and also in total pouch dogs. The relationship between dose of histamine and magnitude of inhibition by a fixed dose of depressant as well as the relationship between magnitube of response to a fixed dose of histamine and magnitude of inhibition by a fixed dose of depressant were studied. The relationship between dose of secretory depressant and magnitude of inhibition was also studied.

Effects of homologous carbamates on the respiration of blocked and active embryonic cells. JOSEPH HALL BODINE AND LAURENCE ROCKWELL FITZGERALD (by invitation). *Zoology Laby., State Univ. of Iowa, Iowa City, Ia.* A study was made of the effects of an homologous series of carbamates (ethyl n-methyl, ethyl, n-butyl, n-propyl, n-amyl, iso-amyl) on the oxygen uptake of mitotically active and blocked embryos of the grasshopper, *Melanoplus differentialis*. No significant differences in response to these reagents have been found for active and blocked embryonic cells. In general, all reagents produce a marked inhibition in the oxygen uptake. Ethyl carbamate in low concentrations seems to produce a marked and consistent stimulation while all other compounds tested by the present methods did not produce such results. A discussion of the present results, in view of the many existing theories of narcosis, is given.

Influence of hesperidin methyl chalcone on blood flow and secretion of the salivary gland. DAVID F. BOHR and W. JOHN KENFIELD (introduced by Robert Gesell). *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* This study of the influence of hesperidin methyl chalcone (HMC) on the rate of salivary secretion and of blood flow through the submaxillary gland of the dog has been directed towards an understanding of the cause of vascular changes produced by this drug. One mg. HMC/kg. body weight injected into the internal carotid artery did not alter the rate of blood flow or of salivary secretion of the gland. The same amount of HMC injected into the external carotid artery supplying the gland caused a striking increase in blood flow through the gland but did not alter rate of salivary secretion. These local injections had no influence on the secretory or blood flow changes produced by stimulation of the chorda tympani or the sympathetic trunk innervating the gland. Similarly the vasoconstrictor and secretory actions of epinephrine were still evident after the administration of HMC. Autonomic blockade with tetraethyl ammonium chloride did not change the vasodilator action of HMC. It would appear then,

that this vasodilatation is the result of the local action of HMC on the vascular musculature. Neither a central nervous system action nor a peripheral one on the autonomic nerves supplying the submaxillary gland was demonstrated.

Effect of choline and inositol on hypercholesterolemia and hyperlipemia in the cholesterol-fed chick. C. BOLENE (by invitation), J. STAMLER (by invitation), R. HARRIS (by invitation) AND L. N. KATZ. *Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago, Ill.* In experiments designed to determine the biochemical parameters of the atherosclerotogenic process, we have undertaken studies on factors affecting the plasma content of cholesterol, phospholipids and fatty acids. Addition of 2% cholesterol to the normal diet of the chick results in a hiperlipemia involving all lipid elements. The degree of development of atherosclerosis is in general related to the degree of increase in these constituents in the plasma. Addition of lipotropic factors to such cholesterol-rich diets in the form of 1% choline plus 1% inositol resulted in a more marked hypercholesterolemia and hyperphospholipemia. This effect is progressive with values at 1, 3, 5 and 8 weeks increased to levels about 50% above the non-lipotrope-fed birds. This occurred despite the fact that there were no significant differences in food intake and weight gain. These results suggest that these lipotropes act to mobilize liver lipids and produce a prolonged transport hyperlipemia. Choline and inositol failed to modify or prevent atherosclerosis in birds killed after 15 weeks or diet.

Experimental studies in ascorbic acid metabolism. WALTER M. BOOKER, RAYMOND HAYES (by invitation) and MARIANNA SEWELL (by invitation. *Depts. of Pharmacology and Oral Medicine, Howard Univ., Washington, D. C.* Clinical studies underway in this laboratory on ascorbic acid metabolism and its relation to certain gingival disturbances have provoked many new experimental approaches to the problem of ascorbic acid metabolism and the effects of ascorbic acid on the metabolism of other substances in the animal body. In unanesthetized and anesthetized dogs ascorbic acid administered at the rate of 100 mg. hourly for 3 hours rises rapidly in the plasma and slowly in the cells. Only after the second hour is a significant increase noted in the cells. This suggests a barrier between the plasma and the cells. There is some evidence that the adreno-cortical hormone can lower the barrier, causing a more rapid rise in the cells. In both unanesthetized and anesthetized dogs, ascorbic acid administered either hourly or by constant drip causes interesting changes in the electrolytes of the blood, such as a fall in chlorides, in sodium and a rise in potassium. Urinary chlorides and sodium rise, while potassium falls. Adreno-cortical extract, administered at intervals while ascorbic acid is being given, causes a decrease in the urinary sodium and chlorides, while these substances may rise in blood toward the control or beginning levels. In some instances, under the influence of the adreno-cortical hormone, the urinary chlorides decrease without significant increase in serum chlorides. Thiocyanate space and blood volume studies suggest movement of chlorides to extracellular spaces. Evidence has been obtained showing ascorbic acid in high quantities causes a diminution of blood cholesterol and of cholesterol at the adrenal gland, where greater the amount of ascorbic acid, the less the cholesterol. Adreno-cortical hormone can increase adrenal storage of ascorbic acid.

Localization of the central nervous mechanism of emesis. H. L. BORISON (by invitation) and S. C. WANG. *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* By means of electrical stimulation with the Horsley-Clarke stereotaxic instrument, the vomiting center has been functionally localized in the dorso-lateral portion of the lateral reticular formation of the medulla oblongata of the cat (Borison and Wang, to be published). This finding is of particular significance since it has been shown that responsive loci for salivation (Wang, 1943), spasmodic respiratory movement (Borison, 1948), vasomotor effects (Wang and Ranson, 1939), and sustained inspiration (Pitts et al, 1939) are situated in areas immediately adjacent to that localized for emesis. Several dogs have been prepared with chronic lesions in the medulla for the purpose of dissociating the component activities involved in emesis. Preliminary experiments indicate that with lesions up to 30-day duration the emetic response to apormorphine is either seriously impaired or completely eliminated. Histological study has not yet been carried out on any of the tested preparations but gross examination of the brains of animals which did not survive the procedure indicate that the lesions are placed approximately in the dorso-lateral portion of the lateral reticular formation. Attempts will be made to correlate the degree of impairment of emesis with the size and position of the lesions.

Renal function in untreated adrenalectomized rats. W. R. Boss (by invitation), JAMES H. BIRNIE (by invitation) and ROBERT GAUNT. *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.* Studies of renal function in salt-maintained adrenalectomized rats indicate that renal clearance is essentially normal (Lotspeich; Friedman *et al.*). Water excretion tests, however, indicate serious alterations in renal function. Creatinine clearance studies were conducted on untreated fasted female normal and adrenalectomized rats weighing approximately 175 gm., following methods used by Lotspeich. Ninety-six rats, divided equally between the various groups studied, were used in this investigation. The glomerular filtration rate (C_{Cr}) of normal rats given

5 cc. of water by stomach tube was .820 cc/100 gm/min. and the urine flow (V) was .022 cc/min. Eighteen hours after adrenalectomy the C_{Cr} was .686 and the V was .015. Five days after operation the C_{Cr} was .551 while the V was .013. When adrenalectomized rats were subjected to moderate and high water loads, the disproportionate fall in glomerular filtration rate and urine excretion rate was even more striking. When two hourly doses of water were administered (3% body surface/dose) the C_{Cr} of the dummy operated controls was 1.011 and the V .083; in rats adrenalectomized for 18 hours the C_{Cr} had fallen to .759 and the V to .041; 7 days after adrenalectomy the C_{Cr} was decreased to .701 with a V of .008. When 4 hourly doses of water were administered the C_{Cr} of the dummy operated controls was 1.010 and the V .118; 18 hours after adrenalectomy the C_{Cr} had fallen to .590 and the V to .031; 7 days after operation the C_{Cr} was down to .447 with a V of only .006. These results show that the rate of urine flow falls in all cases more than the rate of glomerular filtration. This indicates that in untreated, in contrast to salt-treated adrenalectomized rats, a decreased glomerular filtration may contribute to loss of water diuresis, but that an increase in tubular reabsorption of water is probably a more important factor.

A method for analyzing dog muscles for myoglobin. WILLIAM J. BOWEN. *Laby. of Physical Biology, Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* A method has been devised for analyzing dog muscle for myoglobin (Mb) in the presence of hemoglobin. Cleaned muscle is pulverized at the temperature of dry ice as described by Poel (*Science* 108: 390, 1948). An exactly known quantity (about 1 gm.) of the muscle powder is washed into a stainless steel homogenizer tube with a carefully measured volume (about 20 ml.) of M/40 phosphate buffer, *p*H 5.9. It is thoroughly homogenized and then centrifuged at 1000 *g* for 10 minutes. The supernatant is heated to 56°. As soon as discrete particles appear, the extract is rapidly cooled and then centrifuged at 1000 *g* for 10 minutes. It is filtered through fast paper from which it emerges as a clear red solution. This is adjusted to *p*H 6.5 with solid K_2HPO_4 and then gassed with CO in a rotating tonometer. To insure complete conversion to MbCO, $Na_2S_2O_4$ is added to the tonometer after 10 minutes of gassing. After a total of 12 minutes of gassing the density (log Io/I) of the solution is determined at 538 and 568 mμ. The molar concentration of Mb is calculated by substitution of the densities and the appropriate extinction coefficients for MbCO and HbCO in the Vierordt equation (*Ann. Rev. Biochem.* 10: 509, 1941). The amount of myoglobin/gm. of muscle is calculated by using the molecular weight 17,300 (Bowen, *J. Biol. Chem.* 176: 747, 1948). By this method 9 samples of one heart, having an average of 3.60 mg. Mb/gm. of muscle, yielded a $\sigma = 0.28$ and a coefficient of variation = 8%. Also, a known quantity of dog myoglobin added to muscle homogenate was recovered 100%.

Hypoxia of 18,000 feet simulated altitude and myoglobin content of dogs. WILLIAM J. BOWEN. *Laby. of Physical Biology, Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* The left anterior trapezius muscle of 7 dogs was removed by biopsy and analyzed for myoglobin content by the method described in preceding abstract. The dogs were then exposed to 18,000 ft. simulated altitude 6 hours per day, 6 days per week. Hematocrit and hemoglobin values and changes in body weight were followed as criteria of acclimatization. After 5 to 6 months of exposure the dogs were sacrificed and the trapezius muscle from the other side analyzed for myoglobin content. Final hematocrit and hemoglobin values were 119 to 170 and 122 to 170%, resp., of initial values. Body weights in each dog increased during the first month of exposure and then remained nearly constant. The myoglobin contents before and after exposure were as follows:

	Mg. Mb/gram of wet muscle	
Dog	*Before Exposure*	*After Exposure*
1	3.27*	2.80
2	4.70*	4.33
3	5.20*	4.73
4	4.60	4.20
5	4.42*	4.62
6	5.07	3.75
7	3.80	4.22

These values are means obtained in repeated or duplicate analyses, except those marked (*). One value deviated 11% from the mean; another 8%. The remainder deviated 5% or less. The results indicate that exposure to simulated altitude of 18,000 ft. causes no increase in the Mb content of muscle in contrast to the response of the hemoglobin of blood.

Principles of design and operation of internal standard flame photometers for sodium and potassium determination. ROBERT L. BOWMAN and ROBERT W. BERLINER (introduced by DAVID P. EARLE, JR.). *New York Univ. Research Service, Goldwater Memorial Hospital, New York City.* Construction of two internal standard flame photometers for sodium and potassium has led to improvements in design and technique of operation The basic aims have been simplicity of design and establishment of operating conditions in which minor variations of gas and air pressures, atomizing rates, etc. are maximally compensated by parallel changes in the output of cells measuring light from unknown and internal standard. Critical mechanical and electrical components were evaluated for their contribution to stability and accuracy, and extensive study made of parameters determining stability and independence of external variables.

Optimum operating conditions must be evaluated individually for each instrument by determining, separately, for sodium, potassium and lithium, a number of curves relating electrical output to atomizing rate, gas and air pressure, and ion concentration. Operating conditions are selected so that, as nearly as possible, changes in any of these will produce no effect on reading or be maximally compensated by the internal standard. Changes in gas and air pressures which may effect readings are corrected by resetting, emphasis being upon indication with sensitive gauges rather than automatic pressure regulation. Application of these principles to other instruments has been reported to be of material aid. With the present instruments over 6000 and 9000 determinations, in 14 months and 2 years respectively, have been made without interruption for repairs. Accuracy attainable with single readings of unknowns is ±1.0 and 2.0 μeq/l for potassium and sodium respectively in the diluted sample. No interference from other substances in solution is detectable.

A new method of fibrin determination and applications to study of the thrombin-fibrinogen reaction. PAUL W. BOYLES (by invitation) and JOHN H. FERGUSON. *Dept. of Physiology, Univ. of North Carolina, Chapel Hill.* Determination of fibrin by the biuret method (J. Fine, *J. Lab. Clin. Med.* 24: 1084, 1936) was modified by use of Mehl's (*J. Biol. Chem.* 157: 173, 1945) reagent, photoelectric colorimetry, and calibration of colorimetric readings against both gravimetric (fibrin dry weight) and macroKjeldahl (fibrin N) determinations. A commercial (Armour's) bovine plasma fraction-I, analyzing 50% fibrinogen (clottable), after reprecipitation at 2–5°C. with ammonium sulfate at 25, 20, 20% saturation, successively, with subsequent dialysis, yielded fibrinogen solutions 96.7% clottable, with only minute traces of prothrombin, and no fibrinolytic protease. Clotting-times and fibrin yields were studied with variations of: *1*) fibrinogen concentration, *2*) thrombin concentration (purified bovine thrombin), *3*) temperature, *4*) *p*H, *5*) NaCl content, *6*) Ca ion content, *7*) added colloid (acacia), *8*) heat (48°, 12 min.) induced profibrin, *9*) time after clotting (at 37°C.). After 48 hr. the fibrin yields were practically identical (aver. over 95%) independent of the above variables, except for inhibitory concentrations of NaCl and $CaCl_2$, which reduced yields significantly (e.g. 65% in 0.5 M $CaCl_2$). Clotting-times were much modified by these variables. It is particularly significant that the 48-hr. fibrin yield was unaltered by varying the thrombin dilution from 1:1 to 1:250, despite a range of clotting-times, for the series, of 10 to 190 seconds. It is concluded that the thrombin-fibrinogen reaction does not follow any simple stoichiometric law but resembles the reaction kinetics of a colloidal enzymatic reaction.

Adsorption of urinary gonadotrophin on kaolin. JAMES T. BRADBURY, ELWYN BROWN and WILLIS E. BROWN. *Dept. of Gynecology, Univ. of Louisville, Louisville, Ky., and State Univ. of Iowa, Iowa City, Ia.* The following method has been devised for recovery of gonadotrophic hormones. Its main advantages are: *1*) reduction in toxicity of the extracts and *2*) a marked saving the amount of alcohol needed.

The urine of a 12- or 24-hour specimen is adjusted to *p*H 4.5 with acetic acid. Add 5 volumes % of kaolin (powdered and acid washed in a 20% aqueous suspension), stir thoroughly and then allow to settle. Decant supernatant urine. Centrifuge and discard supernatant. (Kaolin may be resuspended in water one to three times to wash it free of urine.) Add normal ammonium hydroxide in a volume equal to that of the original kaolin suspension used and stir thoroughly. Centrifuge and re-extract with a second volume of ammonium hydroxide. Combine the ammonia extracts, add acetic acid to *p*H 8.5 and centrifuge. Save the clear supernatant, adjust to *p*H 5.5 and add 4 volumes of 95% alcohol slowly while stirring. Set in refrigerator several hours to complete the precipitation. Centrifuge and dry the precipitate.

The dry precipitate is dissolved in water or phosphate buffer *p*H 8.0 and the solution injected into immature rats or mice for assay. The recoveries of gonadotrophins from pregnancy or castrate (postmenopausal) urines have been quantitative when compared with those obtained by the usual method of adding 4 volumes of alcohol to the original urine specimen.

Analysis of mosquito tissues for sodium and potassium and development of a physiological salt solution. SARAH BRADFORD (by invitation) and ROBERT W. RAMSEY. *Dept. of Physiology and Pharmacology, Medical College of Virginia, Richmond, Va.* The midgut and Malpighian tubules of Aedes egyptii were analyzed for their content of sodium and potassium. The tissues from 1000 mosquitoes were pooled to provide sufficient Na and K for one analysis. A total of 6 such analyses were done. The Na and K content of the midguts and Malpighian tubules of 1000 mosquitoes kept on a normal diet averaged 6.91 mg. Na/gm. dry weight of tissue and 15.6 mg. of K/gm. dry weight. Both the sodium and potassium contents decrease with time on a salt free diet until after two weeks sodium is reduced to 2.5 mg/gm. dry weight and potassium to 10.4 mg/gm. dry weight. Malpighian tubules have a lower potassium content than midgut. No differences in sodium or potassium content due to age or sex of the mosquitoes were noted.

A physiological salt solution having the following composition was developed for mosquito tissues: NaCl-128 mM/l., KCl-4.7 mM/l., $CaCl_2$-4.0 mM/l., M/150 potassium phosphate buffers to *p*H 6.8. Ex-

cept for the added buffer (which contributes 11.34 mM/l. of K) this solution is the same as that which Bodenstein previously found best for Drosophila. This solution will maintain in the cold, a mosquito heart preparation alive and beating for as long as 280 hr. If small amounts of the B vitamins or p-amino-benzoic acid are added to the above the average survival is prolonged but the maximum so far found (300 hr.) is about the same. Mosquito hearts can survive for as long as 30 hr. in salt solutions that are very high in potassium and low in sodium provided sufficient magnesium is present.

Light tonus of the human eye. G. A. BRECHER (introduced by C. J. WIGGERS). *Dept. of Physiology, Univ. of Prague, Czechoslovakia, and Dept of Physiology, Western Reserve Univ. Medical School, Cleveland, Ohio.* The tonus of the eye muscles and thus the muscular balance of the nonfixating eye is maintained by various reflexes, among which light tonus plays an important rôle in lower animals. No such light tonus has been proven for the human eye. The influence of light upon the heterophoria of the nonfixating eye was measured in 21 normal subjects with a new method permitting the continuous recording of heterophorias under varying optical conditions. The nonfixating eye displays a greater hetophoria upon diffuse illumination than in the dark. The increase of the eye deviation is greatest immediately after the light exposure, and reaches its maximum in about 2 minutes, after which time no appreciable increase can be recorded. The heterophoria increase/time ratio follows the course of a parabola. Upon discontinuation of the illumination the hetophoria decreases to the same value which it previously had in the dark. The degree of the heterophoria is directly proportional to the light intensity. Illumination of the fixating eye causes also a heterophoria increase of the nonfixating eye, indicating the consensual nature of the mechanism involved. It is assumed that the heterophoria increase upon illumination of the retina is due to the existence of a light tonus which contributes to maintain the muscular balance of the nonfixating eye.

Chronic toxicity studies on hydroquinone. N. R. BREWER (by invitation) and A. J. CARLSON. *Physiology Laby., The Univ. of Chicago, Chicago, Ill.* Hydroquinone added to food fats in the amount of 0.01% decreases the rate of oxidative rancidity from 2 to 50 times, thus reducing food deterioration and food spoilage. Is this amount injurious to the health of man or animals? The following tests have been run to answer this important question. *1*) Twenty weanling rats, started on a diet containing 0.25% hydroquinone, showed no evidence of toxic effects (growth, reproduction, autopsy findings) after 2 years on test when compared to 20 controls. Twenty offspring of these rats were continued on this test for a year with no signs of toxic effects. Twenty rats were fed a diet containing 1.0% hydroquinone. After 2 years the only finding was a slight decrease in the rate of growth as measured by body weight. Fourteen rats were fed a diet containing 5.0% hydroquinone. The rats consumed very little food, lost weight rapidly, and after 9 weeks some of them developed a blood condition resembling aplastic anemia. *2*) Dogs refused to eat food (apparently because of the bitter taste) containing more than 0.02% hydroquinone in the total diet. By placing the hydroquinone in sugar coated tablets in the food, one dog consumed 16 mg. hydroquinone/kg/day for 34 weeks, without any evidence of injury when compared with 3 control sibs. Sixteen mg/kg/day is a 160-day supply/day, or a 100-year supply for 34 weeks, for man, if man were to ingest 1 gm. fat/kg/day and all of the fat were treated with 0.01% hydroquinone. *3*) Cats appear more susceptible to hydroquinone. But 10 mg. hydroquinone/kg/day for 6 weeks showed no untoward symptoms to date, when given to 1 of 4 young sibs, and 15 mg/kg/day for 6 weeks showed only a small decrease in the growth acceleration curve when given to another sib. Fifteen mg/kg/day is a 150-day supply per day, a 17-year supply for 6 weeks, for man, if man were to ingest 1 gm. of fat/kg/day and all of the fat were treated with 0.01% hydroquinone.

Factors influencing contractile force of the heart in open-chest mammalian experiments. H. H. BRILL, M. DEV. COTTON and R. P. WALTON (introduced by T. G. Bernthal), *Dept. of Pharmacology, Medical College of South Carolina, Charleston, South Carolina.* Contractile force of a section of the right ventricle was measured directly by means of variable spring tensions inserted in the system of levers of the Cushny myocardiograph typically attached in open-chest preparations. All experiments were carried out on vagotomized dogs, with pericardium opened and with high oxygen concentrations in the mechanical respiratory system. Compensation for changes in heart size were accomplished in two ways *1*) by preliminary calibrations which determined the effect of measured changes in position of the collar which mechanically protected the muscle section from being stretched beyond the usual point of diastolic relaxation *2*) by adjustment of this collar as heart size changed. (This latter has been facilitated by construction of a micrometer screw adjustment mechanism.) Moving and re-setting this collar over wide ranges demonstrated that the muscle section could be stretched to approximately twice its diastolic resting length with an increase in contractile force several hundred percent greater than that of the control period. Within this range, progressive increments of stretch produced progressive increments of contractile force in a linear relationship. Progressive increments of venous

pressure obtained by massive saline infusions and progressive decrements of venous pressure obtained by venous hemorrhage resulted in moderate changes in contractile force which, in a few experiments, followed the linear relationships of Starling's Law but for the most part appeared to be substantially affected by other factors. Partial occlusion of the inferior vena cava lowered venous pressure, contractile force and arterial pressure, the effects apparently operating in close association. Partial occlusion of the pulmonary artery and of the aorta produced, in each case, only limited effects. Sudden release of constriction in the vena cava, the pulmonary artery or the aorta frequently produced an immediate transient increase in contractile force and stroke amplitude distinctly greater than that of the control period. Substantial decreases in contractile force (30–60%) were obtained by ligation of coronary arteries supplying the area of lever attachments.

Gravity and applied accelerations. S. W. Britton and O. B. Murray, Jr. (by invitation). *Physiological Laby., Univ. of Virginia, Charlottesville, Va.* The effects of gravitational force on the circulation have been observed to be similar to those exerted by a force of 1 *g* imposed on an animal on a centrifuge with 10-ft. rotor arm. ECG, arterial blood pressure, heart rate and other determinations were made over periods up to one hour with forces directed from head to foot and *v. v.*, or positive and negative *g*.

Compared to the influence of 1 *g* applied by centrifuge or tilt-table continuously for 30 minutes, dogs showed similar resistance to 3–4 *g* applied discontinuously or alternately, 5 seconds on and 5 seconds off, over the same period. On the basis of *g*-minutes employed, it is noteworthy that the organism withstands about twice as much stress (in force-time) in the latter as in the former case, or 45–60 *g*-minutes compared to 30 *g*-minutes.

Discontinuous forces even as high as 6 *g*, 5 seconds on and off, were well withstood for periods of 30 minutes. Quadrupeds are nevertheless much less resistant to gravitational and high accelerator forces than primate animals and man.

Effect of hypothermia on bromsulphalein removal. Radford Brokaw (introduced by K. E. Penrod). *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* In normothermic dogs under moderate sodium pentothal anesthesia the percentage disappearance rate of intravenously injected bromsulphalein is not constant, but is nevertheless prompt and rapid. Sixteen anesthetized dogs were injected at normal body temperature with bromsulphalein (5 mg/kg.). Blood samples at 6 minutes showed an average level of 1.85 ± 0.93 mg/100 cc. of plasma, and 12 minute samples averaged 0.65 ± 0.20 mg/100 cc. or 35% of the 6-minute level. Following this marked initial fall there was a more gradual decline in the concentration of bromsulphalein so that by 30 minutes 0.314 ± 0.11 mg/100 cc. remained, or 17% of the 6-minute blood level. When 7 anesthetized dogs were immersed in an ice bath and the injection given intra-arterially at 35° C. rectal temperature, the average plasma level after 6 minutes was 4.53 ± 1.44 mg/100 cc. In 12 minutes the average plasma level was 2.89 ± 1.62 mg/100 cc. or 64% of the 6-minute level. Ten dogs received the dye injection at 30° C. rectal temperature. Their 6-minute levels averaged 4.82 ± 1.25 mg/100 cc., with the 12-minute samples showing 3.24 ± 0.93 mg/100 cc., an average of 67% remaining. Both series of hypothermic dogs showed a marked retention of the dye after 30 minutes. In the 35° C. group an average of 1.68 ± 1.03 mg/100 cc. was obtained. These values represent 37% and 43% of the dye remaining respectively from the 6-minute plasma levels as contrasted with only 17% remaining in the normothermic controls. These data indicate that hypothermia decreases both the quantitative and relative amounts of bromsulphalein removed.

Temporal relations in the auditory pathway. John M. Brookhart and Harlow W. Ades. *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, Ill., and the Dept. of Anatomy, Emory Univ. School of Medicine, Emory University, Ga.* Unipolar recordings of responses from various stations along the auditory pathway have been recorded using anesthetized cats. Monaural and binaural click stimuli were furnished by brief condenser discharges activating hearing-aid receivers.

Introductory deflections, preceding the principal responses from the cortex, radiation and geniculate, occur at the same time as discharges from the inferior colliculus and are possibly due to electrotonic conduction from mid brain levels.

Latencies of responses to monaural stimulation from a single animal typical of the series are given below in msec.

	AUD. CORT.	AUD. RAD.	MED. GENIC	BR. INF. COLL.	INF. COLL.	TRAP. BODY	AU TU-BER.
Ipsi.	7.5–8.0	6.0–7.0	5.5–6.0	3.5	3.5	2.0	1.0
Contra.	7.5–8.0	6.0–7.0	5.5–6.0	2.5	2.5	2.0	1.5

The lapse of 2.0 msec. between the geniculate and cortical responses, and of 2.5–3.5 msec. between the brachial and geniculate responses suggests either multiplicity of synapses or unusually long nuclear delays at these points. The geniculate response is similar in crest time and duration to the cortical response and seems to be unaffected by removal of the ipsilateral cortex. The differences in the latencies of ipsilateral and contralateral responses at mid brain levels suggest that one more synapse

is involved in the ipsilateral pathway. The significance of the findings with respect to the functional anatomy of the auditory system are discussed.

Analysis of the mechanism of diurnal rhythmicity in crustacea. FRANK A. BROWN, JR. and H. MARGUERITE WEBB (by invitation). *Dept. of Zoology, Northwestern Univ., Evanston, Ill. and Marine Biological Laby., Woods Hole, Mass.* A well-defined daily rhythmicity of color change occurs in the fiddler crab, *Uca pugnax*. The rhythm persists for many weeks completely in phase with the day-night cycle in conditions of constant darkness, temperature, and humidity. A study was made of the influence of constant illumination, and changes from constant illumination to constant darkness at different times of the day-night cycle, and of alternating periods of light and darkness, different from those of the normal day-night illumination changes. An analysis of the results indicated that the phases of the diurnal rhythm could be altered abruptly by some types of light stimulation administered at certain 'sensitive' periods in the daily cycle, but could not be altered by similar stimuli at other periods. The results appeared, furthermore, to indicate clearly that two centers of rhythmicity were present, each one capable of having its rhythm altered independently of the other, but with one more basic center of inherent rhythmicity capable of influencing gradually a second center having less persistent rhythmicity.

Absorption and excretion of carbon^{14} as CO_2 and bicarbonate. AUSTIN M. BRUES and AGNES NARANJO (by invitation). *Argonne National Laby., Chicago, Ill.* As shown previously, the mouse exposed to $C^{14}O_2$ in a closed system rapidly attains a steady state in which the C^{14} in the animal approximates the amount originally in a volume of air ten times the volume of the mouse. This labile carbon compartment appears to be somewhat greater than the inorganic body carbon, excluding bone carbonate. Excretion rates after a 30-minute confinement period show a small portion of the body C^{14} to be in somewhat more stable combination.

Because of the great rapidity of absorption of $C^{14}O_2$, we have measured the clearance of the tracer gas from air breathed through a tracheal cannula by rats under Nembutal anesthesia. Initially, the expired air contains less than one-half the $C^{14}O_2$ concentration of the inspired air, although the CO_2 concentration is naturally much higher.

To make conditions as favorable as possible for fixation of C^{14}, mice were injected with labelled sodium bicarbonate and allowed to rebreathe expired air for 10 hr. in a 20-liter chamber. Various tissues were analyzed at intervals up to 2 months after removal. It is estimated that less than 2% of the body carbon had exchanged with blood bicarbonate at the end of exposure. Of this retained carbon, about 90% was lost within a week; beyond this time the rate of loss was relatively slow.

Newborn mice receiving C^{14} by exposure of their mothers prior to delivery lose C^{14} much more slowly than adult mice, but the specific activity of their body carbon decreases rapidly due to growth.

Further studies on the pepsin content of histamine stimulated gastric juice. GLADYS R. BUCHER. *Depts. of Biological Sciences and Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* All but two investigators have found pepsin in histamine stimulated gastric secretion. These workers, using acutely vagotomized dogs or cats under chloralose-urethane, report that pepsin output gradually falls to zero in 5 hours of repeated or continuous histamine stimulation. We have attempted to verify their findings, which are contrary to our own on chronic dogs, but with no success. In a dog under nembutal, receiving histamine every 10 minutes, acute vagotomy in the third hour had no influence on pepsin output which remained quite uniform through the ninth hour. The experiment was repeated on another dog, using chloralose-urethane, also with no reduction of pepsin output in 3 hours after vagotomy. There was no reduction in pepsin output in 3 dogs with vagotomized total stomach pouches observed during 7 hours of repeated histamine injections when the standard dose of chloralose-urethane was given in the third hour. In two acute experiments on dogs conducted as described by Bowie and Vineberg, (*Quart. J. Exp. Physiol.* 25: 247, 1935) and three acute experiments on cats conducted as described by Uvnas (*Acta Physiol. Scand.* 4: XI: 1943) no gradual reduction in the pepsin output was observed. In 5 hours, there was a slight tendency for the output to be increased.

The use of fraction I for separation of erythrocytes and leukocytes from whole blood. EDWARD S. BUCKLEY, JR., MARVIN J. POWELL and JOHN G. GIBSON, 2nd. *Medical Clinic, Peter Bent Brigham Hospital and the Dept. of Medicine, Harvard Medical School, Boston, Mass.* The method of Minor and Burnett (*J. Hematol.* 3: 799, 1948) has been modified for separation of red and white cells in quantities as large as 500 ml.

Fibrinogen causes rouleaux formation of red cells, with the result that, in conformance with Stokes' Law, the sedimentation rate of the clumped red cells is accelerated. Since fibrinogen affects white cells little, if at all, their sedimentation rate is not affected. Thus, addition of fibrinogen increases the differential sedimentation rates of the two classes of cells. The *fraction I* used contains citrate, so that no other anticoagulant is needed.

Optimal sedimentation of red cells, and hence maximal recovery of both plasma and white cells, occurs when the fibrinogen concentration of the supernatant is about 6 mg/ml.; the cell-super-

natant ratio is about 0.3; and temperature between 25° C. and 12° C. About 80% of plasma, containing about 80% of the total leucocytes, are obtained after 50 minutes of sedimentation.

The method described offers the possibility of obtaining red cells relatively free from white cells, and white cells free from red cells in large quantities, and hence opens the way for critical studies of the separated formed elements of the blood.

Electrocardiographic observations following explosive decompression of dogs to 30 mm. Hg. BUFORD H. BURCH (introduced by F. A. HITCHCOCK). *Dept. of Physiology, The Ohio State Univ., Columbus, Ohio.* In a previous communication it was reported that dogs explosively decompressed to a terminal pressure of 30 mm. Hg died as a result of ventricular fibrillation which developed about 80 seconds after the explosion. This conclusion was the result of direct observation on the heart through a plastic window sutured to the anterior chest wall. The radical surgery involved may have been a factor in producing the fibrillation, and to test this possibility a series of intact anesthetized dogs were similarly explosively decompressed and continuous electrocardiograms recorded (*lead II*). About 60 seconds after the explosion, changes in rhythm were noted. A-V block, interventricular block and idioventricular rhythms occurred but no evidence of ventricular fibrillation was seen. About 2 minutes after the explosion, a severe slowing of the heart occurred. This is considered significant since previous chest window observations have shown that cessation of the heart beat occurs within 2 minutes after such explosions. Electrocardiographic deflections were observed for as long as 50 minutes after the explosion. A second series of dogs with chest windows was run and electrocardiographic observations made. With this series there was evidence of ventricular fibrillation in about 50% of the cases. It is believed that this surgical procedure tends to precipitate ventricular fibrillation. However electrocardiographic records indicate that a sudden slowing of the electrocardiogram is the most reliable sign of cessation of the heart beat.

Effect of moderate hypoxemia, related to intracardiac venous shunts, on the systemic blood flow. HOWARD B. BURCHELL, BOWEN E. TAYLOR, JULIAN R. KNUTSON and EARL H. WOOD. *Div. of Medicine and Sect. on Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minn.* Determinations of resting systemic blood flow and the effect of exercise on oxygen saturation of arterial blood in 16 individuals, who had moderate hypoxemia related to congenital heart disease, were analysed. The resting values for such saturation in the group averaged 82.4% (range, 75–95). The systemic blood flow averaged 3.68 l/M²/min. (range, 1.1–6.8). No relationship was apparent between systemic blood flow and the resting arterial oxygen saturation. The decrease in arterial oxygen saturation with exercise averaged 22% (range 10–38). The exercise tolerance was not uniformly predictable on the basis of any of the following determinations: the resting arterial oxygen saturation, the oxygen capacity, the resting systemic blood flow, or the decrease in arterial oxygen saturation with exercise. In 2 persons who exhibited, after the initial decrease, a maintained plateau of the arterial oxygen saturation with exercise, the systemic blood flow was determined during exercise. In one, the arterial oxygen saturation decreased from 95–84%, and the systemic flow/100 cc. of oxygen absorbed decreased from 1.9–0.85 l/min. In the second, the arterial oxygen saturation decreased from 86–70% and the systemic flow/100 cc. of oxygen absorbed decreased from 2.3–1.3 l/min. Evident simple relationships were not found between the increased systemic blood flow that may occur with the hypoxemia of congenital heart disease and the other physiologic variables which were studied.

Blood histamine in hypoxia. WILLIAM L. BURKHARDT (by invitation), DON FLICKINGER (by invitation) and HARRY F. ADLER. *USAF School of Aviation Medicine, Randolph Field, Texas.* The blood histamine content of dogs, as measured by biological assay, may increase 10-fold or more in response to continuous hypoxia at a simulated altitude of 18,000 ft. The increase in histamine reached a peak on the 4th day of exposure and may be correlated with the clinical appearance of the 8 dogs which at that time appeared apathetic, lethargic and had an anorexia. However, after the peak had been reached on the 4th day the histamine level began to decline until it reached a control blood level of 0.03 to 0.06 gamma per cc. about 7 or 8 days later which totaled about 12 days of continuous exposure. The decrease in histamine also seemed to be correlated with clinical symptoms in that gradually over the period of the histamine decline the animals lost their apathy and began to eat normally. When the dogs were returned to ground level for 8 days a re-exposure to 18,000 ft. failed to elicit the same marked degree of histamine response noted on the first exposure. However, as compared to the longer continuous exposure, a shorter 4-day exposure to 18,000 ft. followed by only a 3-day rest at ground level and then re-exposure produced an exaggerated blood histamine response. The animals weight, food intake, blood counts and hematocrits have been followed closely during the entire experimental period.

Effect of crude renal extracts and purified renin in experimental malignant renal hypertension. R. O. BURNS, JR. (by invitation), W. H. JASPER (by invitation) and G. E. WAKERLIN. *Dept. of Physiology, Univ. of Illinois College of*

Medicine, Chicago, Ill. Crude hog renal and liver extracts and purified hog renin were injected prophylactically into dogs subjected to simultaneous bilateral renal artery constriction previously found to produce consistent experimental malignant hypertension. Injections were made daily and intramuscularly for 3 months prior to and during survival or up to one month subsequent to renal artery constriction. Crude hog renal cortex extract containing renin was highly effective in protecting against the hypertension, at least partially effective against the arteriolonecrotic lesions and partially effective in prolonging survival time. *4 dogs:* Purified hog renin was only partially effective in protecting against the hypertension and the arteriolonecrotic lesions and in prolonging survival time. *5 dogs:* Crude hog whole kidney extract containing renin did not have an antihypertensive effect, and was only partially effective in protecting against the arteriolonecrotic lesions and in prolonging survival time. *4 dogs:* Crude hog liver extract had no antihypertensive effect and was doubtfully effective in protecting against the arteriolonecrotic lesions of malignant hypertension and in prolonging survival time. *3 dogs:* The mechanism of the antihypertensive effect is not yet determined. Antirenin may be involved. The mechanisms of the protection against arteriolonecrotic lesions and the prolongation of survival time remain to be determined. Present evidence suggests the possibility that the arteriolonecrotic lesions may be due to a renal necrotizing substance which is not renin. (A method which will produce consistent experimental malignant hypertension in the dog has been devised.)

LD-50 studies on hydroquinone. HELEN BURSON (by invitation), N. R. BREWER (by invitation) and A. J. CARLSON. *Physiology Laby., Univ. of Chicago, Chicago, Ill.* In rats the LD-50 of hydroquinone varied with the state of nutrition of the animal and the strain of rats. LD-50 of the Priestly strain was 1317 mg/kg; the Sprague-Dawley strain, 1133 mg/kg; and the Wistar strain, 770 mg/kg.

When the rat is in the fasting state (18 hr. or more without food) the LD-50 is markedly lowered. Variations that are apparent in well nourished rats of various strains become minimized or absent when the rats are fasted. The fasted Sprague-Dawley rats had an LD-50 of 319 mg/kg. The fasted Wistar rats had an LD-50 of 313 mg/kg. Animals in the fasting state show more uniform LD-50s, but this seems not significant in this study, because when added to food fats as an antioxidant hydroquinone will never be consumed in the fasting state.

When fed adult rats are given repeated doses of 500 mg/kg of hydroquinone in propylene glycol by stomach tube the mortality, after 38 administrations, was only 7 out of 20 rats. This is supporting evidence that *1*) the toxic action of hydroquinone is not cumulative, and that *2*) the LD-50 on the fasted animal is not sufficient evidence of the tolerance to this chemical as an antioxidant in food.

Two fasted dogs died after one dose of 150 mg/kg by mouth. Three dogs that were not fasted showed tremors but did not die following a single dose (by mouth) of 200 mg/kg. Four dogs that were not fasted showed similar but more severe symptoms following a single dose (by mouth) of 250 mg/kg. Two dogs were given 28 consecutive daily administrations of 100 mg/kg by mouth with the food, without noticeable effect.

The LD-50 of hydroquinone on cats in the fasting state is between 50 and 100 mg/kg. However, one young cat has to date survived for 6 weeks on 15 mg/kg by mouth, with no symptoms other than a small decrease in the rate of growth when compared to a control sib.

Effects of potassium and lanatoside-C on the failing heart in heart-lung preparations. SANDOR G. BURSTEIN (by invitation), LESLIE L. BENNETT, FRANCIS E. PAYNE (by invitation), and JAMES HOPPER, JR. (by invitation). *Divisions of Physiology and Medicine, Univ. of Calif., Berkeley and San Francisco, Calif.* Dog heart-lung preparations were arranged for the continuous recording of right auricular pressure, aortic pressure, ventricular volume, and left ventricular output minus the coronary flow. Unipolar electrocardiographic tracings were taken directly from the anterior surface of the left ventricle using a wick electrode. When cardiac failure was produced by overloading the heart the administration of 16–32 mg. of lanatoside-C consistently produced reduction in the right atrial pressure and in diastolic ventricular volume, and an increase in cardiac output and in aortic pressure. In four experiments the subsequent administration of potassium (as KCl) reversed the lanatoside-C effects. In five experiments the heart was not overloaded but potassium was administered to the point of production of an increased ventricular diastolic volume. In each case the subsequent administration of lanatoside-C reduced the diastolic ventricular volume. Both potassium and lanatoside-C produced prolongation of the A–V and I–V conduction times as measured from the electrocardiographic tracings. Both substances also produced displacements of the S–T segments and alterations in the T waves. The S–T segment displacements and the T wave alterations produced by a minimum effective dose of one substance could be reversed by the other if it were given in a large enough dose. It was concluded that lanatoside-C can inhibit, delay, or overcome the onset or the course of potassium-induced or overwork-induced heart failure, while high potassium levels are able to inhibit, reverse, or minimize the effects of lanatoside-C.

Alteration of neuron excitability by axon section. BERRY CAMPBELL, EDGAR GASTEIGER and VERNON H. MARK. *Dept. of Anatomy, Univ. of Minnesota, Minneapolis, Minn.* Associated with the chromatolysis produced by axon section is a change in the reflex pattern of the nucleus involved. Previous communications have shown the loss of the proprioceptive component of the segmental reflex, the decrease in the antidromic potential, as well as the continuance of normal conduction in the primary afferent and in the axon of the affected motor cell. This suggests a general decrease in the excitability of the neuron. The study of a pure cutaneous ('flexor') type of response, the tibial-peroneal reflex, during the chromatolytic cycle confirms this interpretation. In a series of 25 cats studied from 2 to 102 days following section of the peroneal nerve the following changes were noted: an increase in central latency, a simplification of the oscillographically recorded reflex potential curve, a decrease in amplitude and, during the height of the cycle, an absence of the reflex. The subliminal fringe, as tested by a second shock, was relatively larger in the early part of the cycle, smaller later. All effects found are consistent with a generalized decrease in excitability of the cells.

Effect of increased intracranial pressure on the circulation in relation to pulmonary lesions. GILBERT S. CAMPBELL (by invitation), FRANCIS J. HADDY (by invitation) and M. B. VISSCHER. *Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* Studies have been made on dogs using right and left heart catheterization to measure pulmonary arterial (PAP) and venous pressures (PVP). Cardiac output, systemic arterial pressure and heart rate were also recorded. Increased intracranial pressure (IICP) was produced by inflating a balloon inserted extradurally through a small trephine opening. It was found that on the average in 18 dogs the mean PVP was 8.2 mm. Hg above intrathoracic before IICP was induced. Afterward the value rose to 18.1 mm. Hg. Concomitant with IICP there was bradycardia, lowered cardiac output and an elevation of PVP and PAP. Gross examination of the lungs at necropsy revealed varying degrees of pulmonary edema, congestion and hemorrhage. Atropine (1.3–1.95 mgm.) was administered i.v. to 4 dogs following IICP. The control mean PVP was 5.5 mm. Hg prior to IICP and the PVP following IICP was 13.9 mm. Hg. The IICP was maintained following the administration of atropine, and the mean PVP fell to 6.6 mm. Hg within 3 minutes. Atropine produced a tachycardia, raised the cardiac output to its control level and apparently protected the animals from the more severe pulmonary edema, congestion and hemorrhage encountered in the earlier group of 18 dogs which were not given atropine. There was no correlation between systemic arterial pressure and these effects.

Effects of an anterior pituitary extract on the metabolism of the liver. JAMES CAMPBELL AND I. W. F. DAVIDSON (by invitation). *Dept. of Physiology, Univ. of Toronto, Toronto, Canada.* The effect of an extract of the anterior pituitary gland on the metabolism of the liver was studied by administering the extract to fasting rats and determining the rate of oxygen uptake and the production of acetoacetic acid by liver slices *in vitro*. These determinations were made at intervals throughout 6 days of fasting and treatment with the extract ($Q^t_{O_2}$ and Q^t_{aa} signify the μl. oxygen taken up and the μg. acetoacetic acid produced, respectively, per mg. total solids of liver per hour). In the 24 hours following the first injection the $Q^t_{O_2}$ was less in the injected than in the control animals; but when recalculated on the basis of fat-free solids ($Q^{ff}_{O_2}$) the values in the pituitary-treated animals were greater. After this 24-hour period both the $Q^t_{O_2}$ and $Q^{ff}_{O_2}$ values were greater in the treated animals. The influx of lipid into the liver produced by the fat-mobilizing stimulus of the pituitary extract accounts for this inversion of the relationship during the first 24 hours. The Q^t_{aa} and Q^{ff}_{aa} values were greater in the injected animals from the start of the experiment. The evidence indicates that a pituitary factor caused an enhanced activity of the fat-metabolising system of liver tissue. The oxygen uptake and the acetoacetic acid production by the liver per 100-gm. body weight were increased by administration of the extract. The addition of octanoate to the liver slices *in vitro* produced an increased oxygen uptake and acetoacetate production; and the extra oxygen taken up in the presence of the substrate could be accounted for by the amount of extra acetoacetate formed. The liver slices from the treated animals had an enhanced ability to produce acetoacetate from the added substrate.

Parathormone and the tubular reabsorption of glucose and phosphate in man. WALTER H. CARGILL and A. CALHOUN WITHAM (introduced by JAMES V. WARREN). *Depts. of Physiology and Medicine, Emory Univ. School of Medicine, and the Medical Service, Lawson Veterans Administration Hospital, Atlanta, Ga.* It is well known that the urinary excretion of phosphate may be increased by the administration of parathyroid hormone and decreased by the ingestion or infusion of large amounts of glucose. We have investigated the role of tubular activity in these reactions in normal human subjects. Glomerular filtration rate was determined by inulin clearance and the rates of reabsorption of glucose and phosphate obtained as the difference between the rates of filtration and excretion. Since the intravenous injection of parathormone (200–400 U) did not alter filtration rate,

the increased excretion of phosphate which followed was due entirely to decreased reabsorption. The reabsorption of glucose usually decreased also. When the rate of glucose reabsorption was increased by the intravenous infusion of 10% glucose solution there was a corresponding increase in phosphate reabsorption, in addition to a decreased rate of phosphate filtration resulting from a lowered plasma level. The injection of parathormone during or immediately following the glucose infusion produced a further increase in the rate of phosphate reabsorption. These observations suggest that the mechanisms of tubular reabsorption of phosphate and glucose are closely related, and that during periods of increased glucose reabsorption the usual effect of parathormone on phosphate excretion may be reversed.

Delay of senescence infertility by dietary restriction. CLAIRE J. CARR (by invitation), JOSEPH T. KING and M. B. VISSCHER. *Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* A group of C_3H female mice have been studied in detail for 23 months. Ten of the original 17 are now living. Since it was known that restriction to 66% of normal calories would disturb but not prevent normal estrous cycles, these mice were restricted to 50% of a normal caloric intake. Protein, minerals and vitamins were not restricted. They remained in anestrus. Occasionally a mouse would show a subestrus response. At two different intervals several mice were given fresh pituitary implants; they responded promptly with a positive vaginal smear. After 14 months half of the group was given increasing caloric supplement with dextrose. Some mice developed positive smears with as little as 0.150 gm. dextrose while others required as much as 1.0 gm. At 21 months the whole group was fed *ad libitum*. Within a week all showed positive smears. At this point they were tested with fertile males. All became pregnant. Some aborted at about the 15th day but subsequently delivered litters. To date the litters have not survived, apparently due to failure of lactation. Observations are being continued. It is apparent that prolonged, severe caloric restriction delays the onset of infertility to a very advanced age. Strain A mice restricted until 8 months old have been studied previously (Ball, Barnes and Visscher, *Am. J. Physiol.* 150: 511, 1947).

Resistance induced by anti-thyroid compounds and by goitrogenic diets against experimental pulmonary edema in rats. K. K. CARROLL (by invitation) and R. L. NOBLE. *Dept. of Medical Research, Collip Medical Research Laby., Univ. of Western Ontario, London, Canada.* ANTU (α-naphthylthiourea) and several closely related compounds have been shown to possess a high toxicity specific for rats. Pretreatment of rats with sub-lethal doses of ANTU induces a marked resistance to its toxic action (Richter 1945, 1947). The present study has demonstrated that resistance to ANTU can also be produced by pretreatment with various other compounds containing the thiourea grouping. Neither the anti-thyroid potency nor the acute toxicity of these substances is directly related to their ability to impart resistance. In addition, resistance to ANTU is found in rats fed on certain goitrogenic diets, especially those containing seeds of the *Brassica* species. This effect has been used as an assay in preparing active extracts of rape seed. Details of the extraction procedure are discussed.

Connective tissue and parenchymal changes in ovary of the rat. H. R. CATCHPOLE and S. C. PAN (by invitation). *Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago, Ill.* Two methods were applied to the study of ovarian connective tissue in immature female rats which were treated with 2–25 RU of equine gonadotrophic hormone and examined after 6–60 hours. Animals of control groups and of groups so stimulated received an injection of 0.5 ml 1.25% Evans blue (intravital) dye intravenously 10 minutes before death. Their ovaries were frozen in liquid air and frozen-dried *in vacuo*. They were then embedded in paraffin, sectioned at 30 mμ, mounted in mineral oil and examined directly. Ovaries from control and gonadotrophin-treated animals were frozen-dried, sectioned at 6 mμ and stained by the McManus-Hotchkiss periodic acid-leucofuchsin technique for glycoproteins. Young adult female rats at different stages of the cycle were also studied by the above techniques. In the normal rat, the extracellular distribution of Evans blue duplicates that of glycoproteins staining with the periodic acid-leucofuchsin method. There is an increased staining density of glycoprotein at pro-estrous and estrous, which is even more pronounced following gonadotrophin stimulation. Coincidentally, more Evans blue is visible in the connective tissue of stimulated ovaries compared either with controls or with normal cyclic ovaries, and the increase is proportional to the dosage or time of action of the hormone. Increased binding of Evans blue and staining density with the McManus-Hotchkiss reagent are interpreted as due to a process of depolymerization occurring in glycoprotein components of the connective tissue. These changes are thought to underlie, or to favor, structural rearrangements within the ovary occasioned by follicular growth.

Studies on bound acetylcholine. DANIEL J. CAVANAUGH and JULIAN M. TOBIAS. *Dept. of Physiology and Toxicity Laby., Univ. of Chicago, Chicago, Ill.* The coexistence of bound acetylcholine (ACh) and cholinesterase (ChE), and, in some cases, of large amounts of free ACh and active ChE (*J. Cell. Comp. Physiol.* 28: 159, 1946) in

tissue stimulates one to ponder why the enzyme does not hydrolyze the substrate *in vivo*. A variety of possibilities exists. There may be a steady state of interaction appearing to be lack of interaction; the enzyme may be in inactive form; the enzyme and substrate may be separated by a structural barrier; one or the other may be held in an inaccessible state, as by adsorption etc. Toward clarifying this situation, preliminary experiments have shown that adsorption of ACh on charcoal can protect it from demonstrably active ChE. It has also been found, using rat brain preparations, that thermal liberation of free ACh from bound precursor is rather rapid, even at relatively low temperatures, being 50% complete in 1 hour or less at 15°C. The rate of unbinding between 15–40°C. is markedly accelerated over that at 0–15°C. Such adsorption protection of ACh from ChE *in vitro* and the relative ease of thermal dissociation of precursor are facts compatible with the notion that bound ACh is loosely held in precursor.

It has also been found that in a distilled water homogenate of brain (rat) the bound ACh is not thrown down at centrifugal speeds something over 20,000 r.p.m. It is either water-soluble or highly disperse, not associated with the denser protoplasmic constituents under these circumstances, and can be shown to be associated with a high lipid content fraction of the brain.

Effect of a psychiatric interview on renal plasma flow and finger skin temperature. JAMES H. CHALMERS (by invitation), ROBERT W. CRANSTON (by invitation), HENRY LONGSTREET TAYLOR and ANCEL KEYS. *Laby. of physiological hygiene, Univ. of Minnesota, Minneapolis, Minn.* Thirty-eight experiments were conducted on 27 well adjusted healthy males and females ranging in age from 20–62 years, all of whom were free of cardiac and renal abnormalities. The subjects were divided into 4 groups on the basis of age and sex. Observations were made of renal plasma flow and skin temperature during a restful control period followed by a period during which emotionally charged topics were intentionally introduced by a psychiatrist familiar with the emotional backgrounds of the subjects. This resulted in moderate emotional tension in most subjects. No marked reactions were noted. A control group was studied by identical methods in which no emotional stimuli were applied. The intraindividual standard deviation of renal plasma flow of the control group (11 experiments on 6 young males) for the periods corresponding to the psychiatric interview was used to calculate T scores for evaluating the response of each of the other subjects to the psychiatric interview. Highly significant changes in renal plasma flow (T score greater than 3) occurred during the interview in 4 of 11 experiments on 7 young males, 5 of 8 experiments on 8 older males and 4 of 8 experiments on 6 females. Positive as well as negative deviations from basal values were found. The degree and duration of emotional response elicited varied between individuals and in the same individual on different occasions. Significant changes in skin temperature of the finger occurred during the interview but were not correlated with changes in renal plasma flow.

Pressure changes and hemorrhage in the middle ear of monkeys resulting from decompression and recompression. HSIANG-TUNG CHANG (by invitation), RODOLFO MARGARIA (by invitation) and SAMUEL GELFAN. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* The pressure changes developed in the middle ear during decompression and recompression have been directly determined by means of a membrane-mirror manometer and recorded photographically. The middle ear pressure increases proportionally with gradual reduction of ambient pressure, the expanding air in the tympanic cavity escaping periodically through the Eustachian tube. The average differential pressure required for forcing the Eustachian tube open in anesthetized monkeys was found to be between 10 to 13 mm. Hg. At the instant of explosive decompression the positive pressure in the middle ear increases suddenly to a value which tends to approach the pressure difference between the pre- and post-decompression altitudes. This excess middle ear pressure, however, is very quickly reduced to that of the ambient pressure.

During recompression anesthetized monkeys are unable to equalize the ambient and middle ear pressures, the negative pressure developing in the middle ear increasing with increasing ambient pressure. This negative pressure, after reaching a critical value, will cause traumatic lesions. Autopsy of explosively decompressed monkeys showed severe bleeding in the tympanic cavity and the mastoid cells of the temporal bone with the ear drum remaining intact. Puncture of the ear drum prior to decompression prevents the tympanic hemorrhage.

Artificial insemination of rabbits and transplantation of rabbit eggs (motion picture). M. C. CHANG and G. PINCUS. *Worcester Foundation for Exptl. Biology, Shrewsbury, Mass., and Tufts College of Medicine, Boston, Mass.* The pictures of Spallanzani (first successful artificial insemination of dogs in 1785), Heape (first successful transplantation of rabbit eggs in 1890), Hammond and Walton (who developed the technique of artificial insemination of rabbits), and Pincus (who developed the technique of transplantation of rabbit eggs) are shown. Methods of rabbit semen collection by means of a piece of rabbit skin and artificial insemination of the rabbit are photographed in color. The recovery of rabbit eggs from excised

oviducts and from the living animal by a surgical method are demonstrated. Living rabbit eggs in the four-cells stage are photographed. Surgical methods for transplanting rabbit eggs at early stages into the oviducts, and blastocysts into the uterus of recipient rabbits are shown. The storage of eggs at low temperatures and the probability of eggs (recovered at different stages, and stored at various temperatures for different lengths of time) developing into normal young rabbits are described. Albino young produced from black chinchilla foster mothers are photographed. A historical review of sperm study and the development of artificial insemination for livestock improvement are shown in a chart. The egg study and the possibility of egg transplantation for the full utilization of female germ cells of valuable animals are illustrated for livestock improvement.

Effect of substrate concentration on the luminescent reaction of Cypridina luciferin and luciferase. AURIN M. CHASE. *Physiological Laby., Princeton Univ., Princeton, N. J., and the Marine Biological Laby., Woods Hole, Mass.* It has been stated (Moelwyn-Hughes, *Ergeb. der Enzymf.* 6: 23, 1937) that the Michaelis constant, K_s, for the luminescent reaction of Cypridina luciferin and luciferase has a negative, and therefore theoretically meaningless, value. For this reason he analyzed and satisfactorilly interpreted this reaction in terms of chain reaction theory. Since the data used by Moelwyn-Hughes represented the velocity constant rather than the initial velocity of the reaction as a function of luciferin concentration, a determination of the relationship between velocity of the luciferin-luciferase reaction and the initial luciferin concentration was undertaken in order to obtain data suitable for analysis in terms of the Michaelis-Menten theory of intermediate enzyme-substrate complex formation. It was found that the initial velocity increases asymptotically to a maximum value as luciferin concentration is increased, yielding data quite susceptible to analysis in terms of the Michaelis-Menten theory. Two enzyme concentrations were used and a 200-fold range of substrate concentration was studied. Excellent agreement with the Michaelis-Menten equation was observed and the calculated value of K_s, the dissociation constant for the luciferin-luciferase complex, was about 6×10^{-7} (the molar luciferin concentration giving ½ maximum velocity.) This order of magnitude is similar to those found for many oxidative enzyme systems which have been studied. The analysis of the data further indicated that one luciferin molecule combines with one luciferase molecule in the formation of the enzyme-substrate complex.

Effect of desoxycorticosterone acetate (DCA) on pituitary content of adrenocorticotrophic hormone (ACTH) after adrenalectomy. CHI-PING CHENG (by invitation), MARION A. SAYERS (by invitation) and GEORGE SAYERS. *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah.* The content of ACTH in the pituitary of the rat is reduced to ⅓ of normal 24 hours after adrenalectomy (Cheng and Sayers, *Proc. Soc. Exptl. Biol. & Med.*, in press). Six of 12 rats were implanted with six 15 mg. pellets of DCA each; 48 hours later, all 12 were adrenalectomized. Twenty-four hours later, the rats were killed and the pituitaries removed. Pituitaries were also removed from untreated intact controls and from 6 intact rats 72 hours after the implantation of DCA (six 15 mg. pellets each). The anterior lobes were frozen and lyophilized and the dried glands of each group pooled and ground to a homogeneous powder. The concentration of ACTH in the powders was determined by the adrenal ascorbic acid-depletion method. DCA had no effect upon the content of ACTH in the pituitaries of the intact rats. The content of ACTH in the pituitaries of the untreated adrenalectomized rats was 15% while that of the DCA-treated adrenalectomized rats was 43% of the control level. In animals with intact adrenals, non-specific stress (scald, sham-adrenalectomy) reduces the ACTH content of the pituitary by 50%. It may be concluded that DCA prevents the marked depletion of pituitary ACTH which normally follows the complete absence of adrenal cortical secretions. The results lend additional support to the concept that pituitary adrenocorticotrophic activity is regulated by the level of cortical steroids in the body fluids.

Order of recovery from excitation in dog, monkey, and human heart; nature of ST-segment and T-wave. H. M. CHERNOFF (by invitation) and L. H. NAHUM. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* The recovery process in mammalian ventricle was studied by *a*) forced extrasystoles, *b*) heating and cooling regions of the dog ventricle, *c*) high altitude anoxia in monkeys and *d*) simultaneously recorded unipolar extremity and precordial leads in man. Practically all normal subjects show in some leads displacement of ST in the same direction as the T-wave. All extrasystoles exhibit similarly displaced ST-segments. High altitude anoxia causes similarly displaced ST-segment and QT shortening. Heating localized ventricular areas produces QT shortening and similar ST-segment displacement. Cooling localized areas lengthens QT and produces ST-segment displacement in same direction as T, but displacement is opposite to that produced by heating same area. ST displacement and T-wave direction in each lead depends upon region of heart whose repolarization has been altered.

In dog, monkey and human heart repolarization is already in progress at end of S. An isoelectric

ST results from balanced repolarization in proximal and distal zones of that lead. The T-wave represents that part of the repolarization process in which the greatest imbalance of potential exists between proximal and distal zone. First stage anoxia causes accelerated repolarization, which is reflected by a specific ST-T pattern. In dog and human heart, the apical and diaphragmatic regions are the earliest to repolarize.

Vasomotor side effects of two new amines which primarily depress intestinal motility. J. R. CHITTUM, F. H. LONGINO and B. H. METCALF (introduced by K. S. GRIMSON). *Dept. of Surgery, Duke Univ., Durham, N. C.* Vasomotor actions of a tertiary amino compound, dibenzylmethyl B-diethylaminopropionate hydrochloride (SC1870), which reportedly has five times the activity of papaverine in relaxing barium-induced spasm have been compared with those of a quaternary amine, ethyl dimethyl B-(9-xanthene carboxylate) ethyl ammonium chloride (SC1703), which reportedly inhibits autonomic ganglia and also has twice the anti-acetylcholine activity of atropine. Five to 7.5 mg/kg. of 1870 or 2.5 to 4 mg/kg. of 1703 delayed gastric emptying and reduced transit time through the ileum of unanesthetized dogs. Essentially similar doses of either drug produced reduction of blood pressure with little change or decrease of pulse rate in dogs anesthetized using chloralose. After either drug the effect of stimulation of distal ends of divided vagi upon heart rate was prevented. During the period of hypotension following either drug, increase of blood pressure normally caused by occlusion of carotid arteries did not occur. Following effective doses of either drug, anoxia failed to increase blood pressure. Stimulation of the central end of a divided vagus nerve after 1870 did produce marked increase of blood pressure. After 1703, however, the pressor response to central vagus stimulation was blocked. After either drug adrenalin increased blood pressure and heart rate. Experiments indicate that both drugs have definite vasomotor effects when given in large doses. These doses, however, apparently were well tolerated when given intravenously to unanesthetized dogs during ileal studies.

Studies of the use of intravenous quinidine orally in certain mechanism disorders. JOHN W. CHRISS (by invitation), MILTON R. HEJTMANCIK (by invitation) and GEORGE R. HERRMANN. *Univ. of Texas Medical School, Galveston.* Quinidine is almost a specific drug for the interruption of serious disorders of the cardiac mechanism such as ventricular tachycardia, atrial fibrillation and atrial tachycardia. In most of our cases the sino-atrial rhythm was reestablished after 1–8 oral doses of 0.2 gm. of quinidine sulfate. In rare cases that proved refractory to oral therapy the seriousness of the patient's condition was such that heroic introduction of quinidine lactate was resorted to. We are fully cognizant of the dangers of using quinidine intravenously and certainly proceeded with great caution. Intravenous quinidine lactate and sulfate were used in several cases of ventricular tachycardia, atrial flutter, atrial tachycardia and atrial fibrillation. The few cases in which even heroic therapy failed will be discussed along with those successfully treated.

The 'motor' cortex of the dog. J. G. CHUSID (by invitation), C. G. DE GUTIERREZ-MAHONEY and FRANKLIN ROBINSON (by invitation). *Neurological Division, St. Vincent's Hospital and Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* The cerebral cortex of 19 dogs under light Dial-urethane anesthesia was stimulated manually using bipolar Ag-AgCl electrodes and a Goodwin stimulator. 'Buried' cortex, exposed by subpial suction of tissue, was explored and found to resemble in its motor characteristics the adjacent exposed cortex. The inferior bank of the coronal sulcus was found to be the superior limit of the 'face' area, while the superior bank of the coronal sulcus was found to be the inferior limit of the 'arm' area. The posterior bank of the presylvian sulcus was found to be the anterior limit of the 'face' area, and in its inferior orbital portion was found to inhibit respiration readily. Although a relatively wide representation for 'face' and its subdivisions was apparent, repeated stimulation of this area and the adjacent posterolateral cortex failed to evoke leg or arm movements. The posterior bank of the cruciate sulcus represented the anterior limit of the 'leg' area. Extension of the 'arm' area onto the anterior bank of the cruciate sulcus occurred in its most lateral portion. Eye movements, pupillary changes and tearing were evoked from the 'buried' cortex of the presylvian sulcus in its most medial (midline) portion. Stimulation of the adjacent cortex lying medially and extending for several millimeters onto the medial aspect of the hemisphere produced the most pronounced pupillary changes; stimulation of the anterior cingulate area inhibited respiration.

Effect of intravenous injection of hypertonic saline on salivary secretion in dogs. L. J. CIZEK (by invitation) and M. I. GREGERSEN. *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* Many investigators have shown that thirst can be induced by the intravenous injection of hypertonic salt solution. Holmes and Gregersen (*Am. J. Physiol.* 151: 252, 1947) demonstrated that the thirst so produced in man was associated with a reduction in the overall salivary secretion. This prompted us to study salivary secretion in the unanesthetized dog before and after the intravenous injection of 20% NaCl (2 cc/kg). Observations were made on 6 dogs, 3 with submaxillary fistulas and 3 with parotid fistulas. Panting in a hot box was used as a uni-

form stimulus. The submaxillary secretion was either unaffected or somewhat augmented by the injection of hypertonic saline. However, the parotid secretion, which was comparatively small but surprisingly constant during the control periods, was sharply reduced or completely absent after saline injection. The difference in response of the two types of glands is unexplained. Furthermore, the evidence so far on the dog is not conclusive in showing that the total secretion into the mouth is reduced by hypertonic saline, although that would be the inference from results on man and from the fact that intravenous saline causes both man and dog to drink.

Responses elicited by combined stimulation of pairs of fixed electrodes in the unanesthetized monkey. GEORGE CLARK and JAMES W. WARD (by invitation). *Dept. of Anatomy, Chicago Medical School, Chicago, Ill., Yerkes Labys. of Primate Biology Inc., Orange Park, Fla. and Dept. of Anatomy, Vanderbilt Medical School, Nashville, Tenn.* Electrodes were implanted aseptically upon the Betz cell area, premotor area and eyefields in each of 10 immature monkeys (*Macaca mullatta*). Selected pairs of these electrodes were stimulated with 60-cycle sine wave current either consecutively or concurrently. As compared to the effects of single stimulation the responses were either unchanged or augmented. In no case did suppression occur. It is suggested that both inhibitory and facilitatory processes may be induced by appropriate cortical stimulation and that under different physiological conditions of the animal, one or the other may be elicited.

Effect of work on renal oxygen utilization. JOHN KAPP CLARK and HAROLD G. BARKER (introduced by J. R. ELKINTON). *Dept. of Medicine and Harrison Dept. of Research Surgery, School of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.* No change in renal oxygen utilization was demonstrated in 15 normal human subjects between basal states and conditions of probably changed renal work, namely saturation of PAH transport mechanism, water diuresis, osmotic (Mannitol) diuresis, 5 subjects each. Arterial and renal venous (catheter) blood was sampled simultaneously. Flow was calculated from the Fick equation using PAH. Oxygen content was determined manometrically and corrected for A-V differences in hemoglobin content. The product of blood flow and A-V difference represents oxygen utilization. 300 gm. of kidney tissue were assumed equivalent to 1.73 square meters surface area. Blood flow remained essentially constant during the tests. The mean control value for oxygen utilization in all 15 patients was 6.1 cc/100 gm/min. (standard deviation ± 2.27, standard error of the mean ± .607). In 12 of the 15 cases no change over 1.5 cc/100 gm/min. was observed.

TEST	OXYGEN UTILIZATION cc/100 GM./MIN.	
	Mean Change	Range
PAH saturation	0.1	(−2.9 to +4.7
Water diuresis	0.7	(−4.4 to +1.2)
Mannitol diuresis	0.4	(−1.0 to +1.4)

Small A-V oxygen differences make the experimental error large. Nevertheless the kidney seems not to vary its oxygen utilization with increased functional activity to a degree comparable to other organs or to the body as a whole where utilization may increase 16 fold during exercise.

Oxidation of CO to CO_2 by the intact animal. ROBERT T. CLARK, JR., J. NEWELL STANNARD and W. O. FENN. *Depts. of Physiology and Vital Economics, and of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Previous experiments with isolated frog tissue in a respirometer containing 80% CO and 20% O_2 indicated that CO in this mixture invariably stimulated gas uptake in heart and skeletal muscle as compared to the rate in air. By direct analyses of the respired CO_2 using radioactive C^{14}, this apparent stimulation was shown by us to be due to the oxidation of CO to CO_2 by the muscles. To determine whether CO would be burned in the intact animal, turtles and mice were placed in concentrations of CO in air ranging from 0.02-0.07% in air-tight containers. The amount of CO oxidized was determined by *1)* measuring by a modification of the Roughton-Root technique the drop in CO concentration in the air during the experimental period and *2)* determining the amount of $C^{14}O$ appearing as $C^{14}O_2$ in the expired air. The gasometric measurements showed that considerably more CO disappeared in the presence of the animals than in the control period. In 10 days the turtles oxidized 52% of the CO present, while mice utilized CO at the rate of 0.044 mm^3/g/min. Two independent measurements on mice with collection of $C^{14}O_2$ indicated the rate of 0.0049 mm^3/g/min. This rate of burning was greater by a factor of 10 than would be expected by extrapolation from the rate observed with isolated tissues in 80% CO and 20% O_2. Extrapolation of these rates to man would indicate oxidation of 20 cc. CO per hour after breathing air containing 0.07% CO.

Occurrence of gastric secretory inhibitor activity in fresh gastric and salivary mucin. CHARLES F. CODE, HENRY V. RATKE (by invitation), GEORGE R. LIVERMORE, JR. (by invitation), and WALTER LUNDBERG (by invitation). *Section on Physiology, Mayo Foundation, Rochester, Minn., and Hormel Inst., Univ. of Minnesota, Minneapolis, Minn.* It was shown some years ago by Brunschwig and his co-workers that the intravenous in-

jection of gastric juice or alcoholic precipitates of gastric juice obtained from patients with achlorhydria produced an inhibition of the gastric secretory response of dogs to a meat meal. This observation was confirmed and extended by Blackburn and one of us (Code). They found that the inhibitor activity was associated with the mucin fraction of gastric juice, and that it was present in samples of commercial gastric mucin, whereas it was absent in preparations of pepsin.

In the present study, fresh gastric mucin was obtained by scraping the mucosa of the stomachs of hogs immediately after killing. The intravenous injection of physiologic saline extracts of this material consistently eliminated the acid gastric secretory response of dogs to rather large doses of histamine given subcutaneously every 10 minutes. The inhibitor activity was found to be precipitated by alcohol. Alcohol precipitates of saliva, like those of gastric mucin, may produce inhibition of the acid gastric secretory response to histamine, but the degree of inhibition produced by salivary preparations thus far has been less than that associated with preparations of gastric mucin. Immediate reactions, manifested often by weakness and a decrease of blood pressure and delayed reaction as indicated by emesis and an increase in temperature may occur when preparations of mucinogenous materials are injected intravenously into dogs. Although inhibition of gastric secretion has been observed in the absence of such reactions, the significance of the occurrence of these reactions in relation to the gastric secretory inhibitor activity of the preparations is not fully understood.

Renal clearances in normal dogs receiving desoxycorticosterone acetate. W. D. COLLINGS, C. F. DOWNING (by invitation) and R. E. HODGES (by invitation). *Dept. of Physiology, State Univ. of Iowa, Iowa City, Ia.* During the course of some studies of renal-adrenal relationships in dogs, it was necessary to investigate the effects of desoxycorticosterone acetate (DCA) on normal dog kidney function. Preliminary studies were made of creatinine and para-aminohippuric acid (PAH) clearances in 3 trained female dogs before, during and after treatment with DCA. Clearances were done by the usual technique of priming the animals first, then maintaining plasma levels of the cleared substances by constant intravenous infusion. Urines were obtained by catheter, and usually three consecutive 15-minute collection periods were run. Rates of urine flow obtained were 2–4 ml/min. The mean control clearances for 79 clearance periods were creatinine, 55.8 ml/min. and PAH, 196.8 ml./min. The mean values for 21 clearances done while the dogs were receiving 2.5–7.5 mg. DCA daily for 5–7 days were creatinine, 66.5 ml/min. and PAH, 191.8 ml/min. Mean values for 26 clearances at daily DCA doses of 10–15 mg. for 6–7 days were, creatinine, 74.8 ml/min. and PAH, 260.3 ml/min. Although other possibilities cannot be ruled out, increased clearances with higher doses of DCA may be related to hemodynamic effects of altered circulating blood volume. On higher DCA doses the classical changes were present, i.e. hemoglobin and hematocrit were reduced whereas both systolic and diastolic blood pressures were slightly elevated.

Cytological effect of slow neutrons. ALAN D. CONGER (introduced by ALEXANDER HOLLAENDER). *Biology Division, Oak Ridge National Laby., Oak Ridge, Tenn.* The operation of chain reacting atomic piles has made a new type of radiation, slow neutrons, available in amounts large enough for considerable biologic effect and possible damage. The biologic effect of the non-ionizing slow neutrons is produced indirectly when they are captured by atoms which then emit the various ionizing radiations. Most important reactions in biologic materials are hydrogen capture of slow neutrons, forming deuterium which immediately emits a gamma ray, and nitrogen capture with the emission of a proton. The action of slow neutrons in causing a specific biologic effect, the breakage of chromosomes, has been studied in the flowering plant *Tradescantia*. Such broken chromosomes are lethal to cells possessing them when those cells subsequently divide; slow neutron exposures of about 15 minutes produced visible aberrations in 50% of the cells. Previous experiments on the same plant have shown that certain types of these chromosome breakage aberrations increase as the dosage squared when x- or gamma-radiation is used, but increase linearly with dose when a radiation of heavy particles is used. The chromosome breakage data from slow neutron treatment given in the Oak Ridge Pile show a linear increase with dose, indicating that the most important reaction of slow neutrons in this tissue is the nitrogen capture with its emission of a proton.

Cardiovascular effects of nembutal, dibenamine and yohimbine on trained dogs. GEORGE CONSTANT (by invitation), ARISTOPHANES ANDRONIS (by invitation) and ERIC OGDEN. *Dept. of Physiology, Univ. of Texas Medical Branch, Galveston, Texas.* Arterial pressure of trained dogs was measured by femoral puncture with a needle connected to a Tycos manometer, the lowest point of the excursion being recorded. Such readings are a few millimeters above diastolic pressure. The drugs were given slowly intravenously in normal saline solutions. Nembutal (30 mg/kg.) greatly increased pulse rate and affected blood pressure variably, a fall being usual. These effects lasted from 1 to 4 hours. Dibenamine (25 mg/kg.) lowered the blood pressure and its effect on the pulse rate was variable; since intravenous dibenamine caused much distress and struggling these pulse rate de-

terminations are doubtful; therefore only 3 such experiments were done. Dibenamine during Nembutal anesthesia produced a further decrease in blood pressure but slowed the pulse rate, not however to the pre-anesthetic level. Yohimbine raised the pulse rate and usually lowered the blood pressure of unanesthetized dogs. Yohimbine given to the nembutalized animal raised both blood pressure and pulse rate. These observations serve again to emphasize the difficulties of interpretation of observations of the cardiovascular system made under anesthesia. The detailed pharmocodynamic interpretation of these differences should not be attempted until a great variety of simultaneous measurements of different cardiovascular functions is available.

Proteolytic activity in cerebro-spinal fluid. GEORGE CONSTANT (by invitation), WIKTOR W. NOWINSKI, ROBERT B. WHITE (by invitation) AND JOY M. HART (by invitation). *Tissue Culture Laby. and Dept. of Neurology and Psychiatry, Univ. of Texas Medical Branch, Galveston, Texas.* Cerebro-spinal fluid from schizophrenic and neurotic patients was investigated for its proteolytic activity. This was measured by the release of tyrosine and tryptophane during incubation for 3 hours at 37.6°C with edestine as substrate and at a final *p*H 4.2. Controls were set up by incubating cerebro-spinal fluid without substrate, but under the same conditions. The estimation of released amino acids was carried out with Folin-Ciocalteu reagent using a Beckman spectrophotometer and the figures obtained were related to a standard expressed in milligrams tyrosine. It was found that in 35 of the 38 cases investigated the amount of released tryptophane and tyrosine in the spinal fluid of these individuals was 40 to 70% greater as compared with the control tubes which did not contain the substrate.

Some methods of producing traveling contraction nodes in adult frog muscle fibers (motion picture). B. A. COOKSON and FLOYD WIERCINSKI (introduced by L. V. HEILBRUNN). *Depts. of Zoology, Univ. of Pennsylvania, and of Physiology, Hahnemann Medical School, Philadelphia, Pa.* Small bundles of muscle fibers, teased from the adductor magnus of Rana pipiens, were immersed in a solution containing 1.3% NaCl and 0.75 vols. % H_2O_2. After approximately 2 minutes small contraction nodes were seen forming in various regions of the fibers. These nodes usually recurred in the same regions at regular intervals. After originating, they traveled along the fibers. Usually the node as it formed split into two nodes traveling in opposite directions. Frequently the nodes collided and canceled out. Sometimes contraction nodes were formed which involved only half the circumference of the muscle fiber. A slight increase in the H_2O_2 concentration produced, instead of traveling nodes, large stationary areas of fiber which rythmically contracted and relaxed. During the contraction the sarcolemma became wrinkled. A slight decrease in the H_2O_2 concentration resulted in complete absence of response. Reduction of the NaCl concentration to .65% (approximately isotonic), also resulted in an absence of response. A solution containing 1.3% NaCl, 0.75 vols % H_2O_2, 1% glutathione and enough 0.1 N NaOH to adjust the *p*H to 7.4 was found to keep its potency for over 2 days, whereas the solution containing only 1.3% NaCl and 0.75 vols % H_2O_2, due to the instability of the H_2O_2, remained potent for only 2–3 hours. With the former solution it was possible to produce traveling contraction nodes in isolated single fibers with injured ends. Preliminary studies indicate that traveling contraction nodes can also be produced by solution of 2-methyl 1:4 napthoquinone in Ca-free frog ringer.

Osmophilia in relation to alkaline phosphatase activity in the chloride cell (gill) of fundulus heteroclitus. D. EUGENE COPELAND (introduced by C. M. WILLIAMS). *Arnold Biological Laby., Brown Univ., Providence, R. I.* Evidence has been previously presented (Copeland, *J. Morph.* 82) of a cell type responsible for the salt excretion described by Smith (*Am. J. Physiol.* 93) as occurring from the gills of marine fish. Subsequently it was suggested (Pettengill and Copeland, *J. E. Z.* 108) that the marked increase in phosphatase activity found when *F. heteroclitus* is adapted to fresh water indicated a physiological reversal of cell polarity, the cell then becoming an absorptive mechanism, accounting for the chloride absorption noted by Krogh (*Z. vergl. Physiol.* 24) in fresh-water fishes. In the present report, a similar marked increase in osmophilia is found to occur as the fish becomes fresh water adapted. Emmel (*Anat. Rec.* 91) has shown a positional relationship of phosphatase activity and Golgi zone in the absorption cells of the intestine. The chloride cell not only shows a reasonable positional similarity of the two phenomena but also a synchronous change in amounts with change in functional activity of the cell.

Ferritin: effect on blood pressure and pressor response in rats. A. C. CORCORAN, G. MASSON and C. SCHAFFENBURG (by invitation). *Research Division and Bunts Inst. of the Cleveland Clinic Foundation, Cleveland, Ohio.* A sample of ferritin was kindly supplied us by Dr. S. Granick. The effects of its intravenous injection were observed in 8 rats anesthetized with pentobarbital; 4 of the animals were bilaterally nephrectomized (to exclude participation of renal V.E.M. in the response) and 4 had experimental renal hypertension (due to silk perinephritis in 1 and to the 'endocrine-kidney' technic of Selye in 3). One carotid artery was cannulated and arterial pressure measured with a mercury manometer. Each experi-

ment consisted of successive injections of ferritin solution (0.1 or 0.2 cc. containing 200 to 400 μg. of ferritin) preceded and followed at intervals of 5 to 10 minutes by injections of adrenaline (0.12 μg., intravenously). Injection of ferritin had no consistent immediate or delayed effects on arterial pressure or adrenaline response. Ferritin or its protein moiety has been identified as V.D.M. and found to inhibit the effect of local adrenaline on the terminal arterioles of the rat mesoappendix for several minutes in a dosage of about 0.005 μg. intravenously (Mazur and Shorr, 1948). From our experiments it seems that these segments of the vascular tree do not participate significantly in the pressor response to intravenous adrenaline. Further, these observations do not support the view that the presence or absence of responsiveness to adrenaline in this segment is closely related to the level of arterial pressure.

Conditions influencing explosive decompression injury. E. L. Corey. *Univ. of Virginia Medical School, Charlottesville, Va.* In an attempt to evaluate the multiple factors operating in conventional experiments on explosive decompression (expansion of intrapulmonary gases, anoxia, aeroembolism), over 300 tests were performed in which rats were placed in a circulating atmosphere of compressed air for varying time periods (2–30 atmospheres; 10 sec.–30 min.), and subsequently decompressed to ambient atmospheric pressure in *c* 0.5 sec. Anoxia was thus prevented, and the effects of rapid gas expansion alone could be observed following exposures to pressure. The effects of anoxia *per se* were studied by placing animals in an atmosphere of nitrogen for suitable time intervals. In rats maintained under positive pressure for 30 min. prior to decompression, 100% mortality was not attained until decompression from 11 atmospheres. In these cases pulmonary lesions were observed in only 15% of the animals, while all exhibited marked aeroembolism. Of 99 animals which succumbed in experiments of this type, 26% gave evidence of pulmonary damage, while 87% showed gross aeroembolism. Obviously time under pressure constituted a potent factor in determining survival after decompression. Thus at 30 atmospheres a time-under-pressure difference of 40 seconds was found to alter the mortality rate from 100% to zero. Aeroembolism thus appeared to be the major cause of injury in these experiments. Anoxia resulted in pulmonary hemorrhage in 50% of animals tested. Results of these tests indicated that anoxic anoxia and aeroembolism far outweigh the physical expansion of intrapulmonary gases as lethal factors in explosive decompression.

Comparative diuretic effects of neutral and alkaline sodium salts in hypoproteinemic edema. Samuel A. Corson and Elizabeth O'Leary (by invitation). *Dept. of Physiology, School of Medicine, Howard Univ., Washington, D. C.* Edema was produced in trained unanesthetized dogs by means of a low protein diet and periodic massive plasmapheresis. Edema (as measured by an increase in thiocyanate space) could be produced by this method within a period of several days and maintained for several weeks. Hypertonic solutions of Na salts were administered intravenously (in the postabsorptive state) by means of a constant infusion pump at the rate of 2.5 ml/min., injecting a total dose of 10 ml/kg. of body weight. The diuretic response was measured until the rate of urine flow began to approach that of the control period. A 1.4M solution of disodium salts of succinate and fumarate increased the rate of urine flow 10-fold (from a control value of 0.5 ml/min. to an average value of 5 ml/min.), this diuresis persisting for about 300 minutes. Subtracting the quantity of fluid injected and the fluid that would have been removed as a result of the normal urine flow, one obtains a figure of 76 ml/kg. as the average net quantity of urine excreted following the administration of the alkaline salts. The injection of either 2M (isosmotic with a 1.4M disodium salt) or 2.8M NaCl (equivalent in Na content to a 1.4 M disodium salt) tripled or quadrupled the rate of urine flow for a period lasting for about 300 minutes. The average net fluid output under these conditions was 24 ml/kg. Thus, intravenous administration of either neutral or alkaline sodium salts leads to the removal of large quantities of fluid in experimental hypoproteinemic edema, the alkaline salts invariably producing a diuresis approximately 3 times as great as that induced by isosmotic or equivalent solutions of NaCl.

Development of Turbulence in Flowing Blood. N. A. Coulter (by invitation) and John R. Pappenheimer. *Dept. of Physiology, Harvard Medical School, Boston, Mass.* The relations between pressure, flow and electrical conductivity of bovine blood were determined over a wide range of flows, corpuscular volumes, and temperatures in glass tubes 2.52 mm. and 6.80 mm. in diameter. The viscosity (calculated from Poiseulle's Law) decreases with increasing flow—a phenomenon known previously to occur in capillary tubes. Presumably change of viscosity with flow is associated with orientation of red cells in the flow stream. This orientation is beautifully demonstrated by changes of electrical conductivity along the axis of the flow stream. At zero flow the (non-conducting) cells are randomly oriented and the electrical resistance is high; as flow progresses, the cells orient to present least hydrodynamic resistance and both the (apparent) viscosity and the electrical resistance diminish in regular fashion toward constant values. Turbulence begins at an apparent Reynolds number of about 1000 as with homogeneous fluids. However, the electrical resistance remains un-

changed even at Reynolds numbers up to 2500. We, therefore, infer that turbulence occurs only in the plasma layer, while viscous flow continues in the central core of oriented cells. It appears possible to consider the apparent viscosity of blood as the resultant of two real viscosities: *a*) the viscosity of plasma alone in the peripheral sleeve; *b*) the viscosity of cells plus plasma in the central core. Evidence suggests that the rate of shear in the peripheral sleeve is so high compared with that in the central core that most of the energy required to maintain flow is dissipated in the plasma layer.

Pulmonary blood flow and alveolar ventilation-perfusion relationship following resection of one lung in man. A COURNAND, R. L. RILEY and A. HIMMELSTEIN (by invitation). *Dept. of Medicine and Surgery, Columbia Univ. College of Physicians and Surgeons, New York City.* Pulmonary blood flow and pulmonary arterial pressure were measured at rest in 9 subjects, 14 to 72 years of age, from 1 month to 11 years after resection of one lung. In 6 the same measurements were repeated during moderate exertion. Although the puomonary arterial pressure at rest was but slightly altered, it rose invariably during exercise. This finding is in contrast with observations in normal individuals during much more severe exercise (*Am. J. Physiol.* 152: 372, 1948). The pulmonary artery pressure rise was small in cases where the remaining lung appeared normal on the basis of physiological measurements, while the rate of blood flow exceeded the blood flow through *each* lung of normal individuals during severe exercise. The maximum load which can be taken up by a normal vascular bed was therefore tested. The pulmonary artery pressure rise was larger in individuals where moderate emphysema developed in the remaining lung, although the increase in blood flow was smaller. The relationship between alveolar ventilation and perfusion was studied in these same individuals according to the method presented in these proceedings by two of the authors. They were normal in 6; in the remaining 3 there was evidence of perfusion of poorly ventilated alveoli and/or of ventilation of poorly perfused area.

Effect of a phychiatric interview on the blood pressure response to cold stimuli. ROBERT W. CRANSTON (by invitation), JAMES H. CHALMERS (by invitation), HENRY LONGSTREET TAYLOR, AUSTIN HENSCHEL and ANCEL KEYS. *Laby. of Physiological Hygiene, Univ. of Minn., Minneapolis, Minn.* The blood pressure responses to a 'cold pressor' test of 9 well-adjusted healthy young men 18 to 24 years old were studied on six occasions during a control period of 7 months. The cold test was standardized as to time of events, relation to meals and activity and psychological atmosphere. Blood pressures were measured by means of a sphygmomanometer and stethoscope with the subject supine during a suitable control period and during the immersion of the hand in ice water for one minute. Each subject was interviewed by the psychiatrist for 5 to 8 hours during the 7-month control period to learn his psychodynamics. When preliminary observations were completed, an interview was arranged in which emotionally charged topics were intentionally introduced by the psychiatrist. There was a mild emotional response of hostility and resentment in the majority of cases but no marked emotional reactions. The interview producee variable but small changes in the resting blood pressure. At the end of the interview, the blood pressure response to cold was determined. The grand average of the mean individual increases in the systolic blood pressure during the control period was 16.03 mm. Hg. The mean response to cold after the psychiatric interview was 24.2. This difference was significant at the 1% level.

Sodium and potassium depletion in rats by means of cation exchange resins mixed with food. J. M. CRISMON. *Dept. of Physiology, Stanford Univ. School of Medicine, Stanford, Calif.* Maintenance of negative sodium balance with the aid of ingested cation exchange resins as suggested by Dock (Trans. *Assn. Am. Physicians* 59: 282, 1946) has received little study and few clinical trials have been reported. Intakes of sodium and potassium and fecal loss of these cations from 10 rats were measured every other day for 23 days. After a 9-day control period, 10% by weight of acid-treated −200 mesh Dowex 50, a sulfonated polystyrene resin was added to the diet. After addition of resin 100 gm. of diet contained, by analysis, 30.3 mEq. of sodium and 16.0 mEq. of potassium. On the 18th day sodium in the diet was reduced to 18.9 mEq/100 gm. and potassium to 13.7 mEq/100 gm. During the control period 96% of ingested sodium and 94% of ingested potassium were absorbed from the gut. During high sodium intake, plus resin, absorbed sodium decreased to 70% and potassium to 82% of the amount ingested. On reduced sodium intake, absorbed sodium decreased to 34%; potassium absorption was still 82%. The low sodium diet containing resin was fed for an additional 10 days, and the animals were killed. Plasma sodium varied from 142 to 167 mEq/l. with a mean value of 152. Potassium concentrations were slightly low with a mean of 3.2 mEq/l.; the range was 2.3 to 3.9. Muscle sodium, potassium and chloride were low with the following mean values in mE,/100 gm. of fat-free dry muscle: Na = 6.68 (4.46 − 9.26); K = 36.8 (32.1 − 40.6); Cl = 5.37 (4.65 − 5.98).

Action potential of heart muscle. HOWARD J. CURTIS. *Dept. of Physiology, Vanderbilt Univ. Medical School, Nashville.* By microdissection methods it has been found possible to isolate small,

homogeneous bundles of muscle fibers from the turtle heart in which the fibers are all parallel and there are very few anastomoses between fibers. These bundles are transferred to mineral oil and stimulation and electrical recording is effected by means of microelectrodes. At about 10°C. spontaneous activity is almost absent. When both recording electrodes are on active muscle, the conducted action potential is monophasic, as contrasted with the diphasic action potential of skeletal muscle under the same circumstances. The monophasic wave is followed by a T-wave which may be either positive, negative or diphasic. When one end of the bundle is crushed or treated with isotonic KCl a resting potential of 10 millivolts or more is recorded. The action potential now falls from the level of the resting potential to zero and remains there for as long as 2 seconds and then relatively rapidly returns to the level of the resting potential. Preliminary estimates indicate that the period of depolarization corresponds closely with the period of contraction and with the S-T interval. Thus the initial part of the action potential represents conduction in the individual muscle fiber presumably by the same mechanism as for skeletal muscle, but unlike skeletal muscle repolarization is delayed for a relatively long time. The T-wave is not a conducted response and represents true cellular re-polarization.

Attempted blockade of the adrenotrophic mechanism of the pituitary in starvation. SAVINO A. D'ANGELO. *Dept. of Biology, Washington Square College of Arts and Science, New York Univ., New York City.* It has been demonstrated (D'Angelo, Gordon, and Charipper. *Endocrinology* 42: 399, 1948) that starvation in the guinea pig fires the adrenotrophic mechanism of the anterior hypophysis and causes marked enlargement of the adrenals. Although reciprocal regulatory control between anterior pituitary and adrenal cortex is shown by adrenal depression with cortical hormone overdosage in the fed animal, it appears that in the guinea pig the cortical hypertrophy of starvation cannot be blocked. Female guinea pigs (300–350 gm.) were subjected to acute starvation during which cortical hormone was maintained at presumably high levels in the body fluids by administering subcutaneously aqueous cortical extract (Upjohn) in 3 cc. doses every 3 hours, 4 times daily, for 9–11 days, and by giving cortical extract in the drinking water as well. Each animal received approximately 150 cc. of the cortical extract over the starvation period. Adrenal enlargement occurred with mitosis frequently encountered in the outer fasiculate region of the cortex. Cytological study of the pituitary indicated high rather than suppressed secretory activity. Two marked peripheral effects were observed in the cortical-treated animals, *1*) oliguria and *2*) marked leucocytosis characterized by a pronounced increase in the numbers of neutrophils showing advanced stages of nuclear lobulation. Further studies on the peripheral blood and on the marrow following high cortical dosage are being made.

Effects of steroids on the endocrine system of the starved guinea pig. SAVINO A. D'ANGELO, ALBERT S. GORDON and HARRY A. CHARIPPER. *Dept. of Biology, Washington Square College of Arts and Science, New York Univ., New York City.* In female guinea pigs acutely starved to 40% body weight loss by complete food deprivation (water allowed), the thyroid lost at a lesser rate (18%), the ovary at about the same rate (44%), and the cervical lymph nodes at a greater rate (68%) than did the body. In contrast, the adrenal underwent absolute hypertrophy (60%) with commensurate increase in solid contents. The results obtained following the subcutaneous administration (6–11 days) of various steroid preparations in high daily doses (Progynon B., 600 RU; progesterone, 15 mg.; desoxycorticosterone acetate, 18 mg.; lipoadrenal extract, 1 cc.; aqueous cortical extract, 10 cc.) indicate that estrogen, desoxycorticosterone and lipo-adrenal, but not progesterone, augmented the adrenal hypertrophy of starvation. The solid content of these enlarged adrenals was correspondingly increased and microscopic examination further indicated a true increase in cortical mass. In the fed animal only the aqueous cortical extract affected adrenal weight (by depression) but desoxycorticosterone and aqueous cortical extract both caused involution of the zona glomerulosa in the adrenals of fed animals. A marked difference on the adrenals was found between the steroid vehicles used. Peanut oil given in 1-cc. daily injections for 9–11 days caused marked adrenal enlargement in fed or starved animals, whereas, sesame oil in equivalent or greater amounts was without effect. The various steroids employed did not appreciably influence thyroid and ovarian weights in either the starved or fed animal, and the regression of the lymph nodes in starvation bore no apparent relationship to the adrenal hypertrophy. The involvement of the anterior pituitary in the end organ response to starvation is considered.

Rise in serum precipitable or protein-bound iodine following massive doses of inorganic iodide. T. S. DANOWSKI and J. H. GREENMAN (by invitation). *Dept. of Research Medicine, Univ. of Pittsburgh School of Medicine, Pittsburgh, Penn.* Riggs, Lavietes and Man (*J. Biol. Chem.* 143: 363, 1942) demonstrated that both inorganic and protein-bound iodine is present in plasma. The work of Taurog and Chaikoff (*J. Biol. Chem.* 165: 217, 1946) with rats indicated that, within limits, increases in the intake of inorganic iodide were accompanied by a rise in the organic iodine of the thyroid gland and plasma. The maximum level of

circulating total iodine recorded was 33.6 gamma %, and the changes in the circulating protein-bound iodine, though definite, were quite small.

This report deals with the relationship between the intake of inorganic iodide and the level of circulating serum precipitable or protein-bound iodine found in 4 patients to whom massive doses of potassium iodide (up to 3 gm. daily), or of a saturated solution of potassium iodide (up to 7 cc. daily), had been given during 4 to 18 weeks. This resulted in total serum iodine levels of 2,100 to 18,100 gamma %. Serum precipitable iodine measured by the method of Barker (*J. Biol. Chem.* 173: 715, 1948) slightly modified, rose progressively as to high as 31.7 gamma %. Removal of any traces of inorganic iodide which would yield false high values was assured by obtaining checks on two separate sets of duplicate or triplicate aliquots of precipitated serum, one set of which was washed with 14 and the other with 18 successive changes, 25 cc. each, of double distilled water. Iodism did not develop nor were these high levels of protein-bound iodine associated with hypermetabolism.

Age and osmotic work in urea excretion. D. F. DAVIES (introduced by H. A. SCHROEDER). *Section on Cardiovascular Disease and Gerontology, National Inst. of Health, Bethesda, Md. and Baltimore City Hospitals, Baltimore, Md.* Previously reported results on degenerative changes in renal function with age have indicated a primary vascular defect. The possibility was considered that this reduction in renal function was caused by lowered metabolic rate and decreased work requirement of the kidney in old age. If this were true, the kidney could be said to undergo an atrophy of disuse having no deleterious effect on other body functions. To examine this hypothesis data on basal urea production, tubular reabsorption of urea, urea clearances and blood and urine urea concentrations were examined for their relationship to their contribution to osmotic work in adult men between the ages of 20 and 90. Urea clearance decreased with age at a rate comparable to that of glomerular filtration rate and tubular excretory capacity after the 5th decade. Tubular reabsorption of urea followed a progressive decline during the seven decades studied. Most of this fall is explained by decreased filtration rate. Urea production (excretion) showed no significant correlation with age. Blood urea concentration showed a moderate increase with age. Calculation of renal osmotic work of urea excretion showed that there is an increase in the amount of osmotic work per unit of functioning kidney tissue with increasing age. Unless a significant and consistent decrease in salt intake occurs during the aging process, involutional changes due to decreased work requirement cannot be the cause of the decrease in effective renal blood flow and excretory functions associated with aging.

Theophylline ethylene diamine and renal function in control subjects and decompensated cardiac patients. JAMES O. DAVIS (by invitation) and NATHAN W. SHOCK. *Section on Cardiovascular Diseases and Gerontology, National Heart Inst., National Inst. of Health, Bethesda, Md., and the Baltimore City Hospitals, Baltimore, Md.* Inulin, diodrast and sodium clearances were determined during 3 control periods and during 5 periods after the intravenous administration of 0.48 gm. of theophylline ethylene diamine (TED). In control subjects, an elevation in GFR and Cl_{Na} was sustained for 50–60 minutes. The rise in effective RPF was usually transitory. Statistically, the ratio E_{Na}/Cl_{In} was more variable and of significantly greater magnitude after than before the injection of TED. This finding indicates that increased sodium excretion cannot be regarded as solely the result of augmented GFR and suggests that TED acts upon the renal tubules to decrease sodium reabsorption.

Patients in congestive heart failure were divided into two groups according to the level of Cl_{Na} before TED administration. In the low Cl_{Na} group, GFR was usually within normal limits but effective RPF was consistently low. In the elevated Cl_{Na} group both GFR and effective RPF were reduced. This evidence indicates that a low GFR is not the primary mechanism for salt retention in patients with cardiac failure. The response in GFR and effective RPF in heart failure patients ranged from a negligible elevation to an increase greater than 100%. Unlike the response in controls, the elevation in GFR lasted for 10–20 minutes only; the increase in Cl_{Na} was usually sustained for 50–60 minutes. The lack of correlation between the duration of the elevation in GFR and Cl_{Na} indicates that the sustained increase in Cl_{Na} is the result of decreased tubular reabsorption of sodium.

Role of the adrenal cortex in the abnormal insulin and adrenaline response of hypophysectomized dogs. R. C. DE BODO, S. P. KIANG (by invitation) and I. H. SLATER (by invitation). *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.* It was previously suggested (*Federation Proc.* 7: 116, 1948) that the gradual development of insulin hypersensitivity and resistance to adrenaline hyperglycemia in the hypophysectomized dog might be related to the secondary atrophy of the adrenal cortex and thyroid. Hypophysectomized dogs manifesting maximal insulin hypersensitivity (more than 60 fold) were given potent adrenal cortical extract (ACE). The total dosage, route and time of administration were varied. Administration of ACE both on the previous day and again 4–5 hours before insulin gave the optimal effect. While 1500 DU had little effect, 3500–4000 DU (for 15- to 20-kg. dog)—an amount many times the maintenance dose for

adrenalectomized dogs—produced the following effects: *1*) it elevated somewhat the post-absorptive blood sugar, *2*) after insulin (0.025 U/kg. i.v.) it prevented the fall of blood sugar to the usual low level—hypoglycemic signs did not develop—and markedly accelerated the recovery to post-absorptive levels. It did not, however, alter the rate of the blood sugar drop. Though ACE in massive doses exerted some 'anti-insulin' action, reversion to a normal response was never achieved. In each case the insulin-induced hypoglycemic response in the ACE-treated animal closely resembled the response obtained in the animal 2–3 days after hypophysectomy.

Similar studies with ACE were made on the adrenaline response of hypophysectomized animals showing resistance to the hyperglycemic action of adrenaline. In every instance, the effect was small and in no case was a return to a normal response noted.

Protein utilization in hypophysectomized dogs. R. C. DE BODO, S. P. KIANG (by invitation) and I. H. SLATER (by invitation). *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.* Houssay showed that hypophysectomized dogs when compared with normals have a markedly decreased nitrogen excretion on fasting, but a normal nitrogen output on meat diet. Soskin found that meat-fed hypophysectomized dogs were able to sustain a normal blood sugar and that after fasting recovery from hypoglycemia occurred with meat feeding. Considering the importance of these observations which might suggest a distinction between 'endogenous' and 'exogenous' protein metabolism in hypophysectomized dogs, it was felt that re-examination of the problem was warranted. We have found that hypophysectomized dogs were able to maintain and even increase their post-absorptive blood sugar level and increase their body weight when fed a trypsin hydrolysate of casein (with vitamin supplement) for a period of three weeks. In a second series of experiments when hypophysectomized animals were fasted, prompt recovery of the blood sugar from hypoglycemic levels was noted within one hour after the feeding of the hydrolysate.

During fasting the hypophysectomized dogs showed a decrease in nitrogen excretion to about ⅓ of that noted in normal animals after comparable or even longer periods of starvation. These hypophysectomized animals showed also a diminished output of potassium and phosphate with unchanged serum levels, a further indication of impaired breakdown of intracellular protein. Thus, we have been able *1*) to confirm and extend the previous observation, that while the metabolism of body protein is defective in the hypophysectomized animal, the utilization of fed protein is adequate and *2*) to demonstrate that the conversion of amino-acids to carbohydrate is very rapid.

A constant delivery pipette particularly designed for analysis of small volumes. R. C. DEERING (introduced by H. C. BAZETT). *Dept. of Physiology, Univ. of Pennsylvania Medical School, Philadelphia, Penna.* Direct determinations of cardiac output require special methods for analyzing small blood samples for which a modification of the Guest pipette possesses advantages. The pipette consists of a calibrated glass tube joined at each end by two-way stopcocks. The capillaries of the upper stopcock connect the calibrated tube to *a*) a cup similar to that of the Van Slyke apparatus or *b*) a smaller overflow cup. The capillaries of the lower stopcock connect it to *a*) a sample inlet tube or *b*) a stem having a rubber tip. The pipette serves to introduce a sample into a Van Slyke apparatus after which standard procedure is followed. The stem, supported by a rubber stopper, is placed in the analyzer cup. For analyses of 0.2-ml. samples, gas-free lactic acid is run into the upper cup, the cocks are reversed and the calibrated section washed and dried. The pipette is filled directly from collecting syringes, the blood sample, preceded by a mercury 'cap,' being forced up the pipette section to the overflow cup. The cocks are reversed cutting off a contained volume. The blood, followed by a mercury drop and the lactic acid, is run into the chamber, thus introducing the whole sample. Present experience indicates a greater accuracy of sample-introduction, so that consistency with 0.2-ml. samples is equivalent to that commonly obtained with 1 ml. With 0.5 ml., conditions are improved and the procedure simplified. Accuracy attained will be illustrated with data obtained on samples from the right heart.

Influence of work on the amount of K-strophanthoside stored by muscle. E. C. DEL POZO, G. ANGUIANO (by invitation) and E. G. PARDO (by invitation). *Inst. de Salubridad y Enfermedades Tropicales Mexico, D. F., Mexico.* It is generally thought that striated muscle is able to store only very small amounts of digitalis glycosides. The heart, on the other hand, can take up much greater amounts per gram of tissue. The cause of this selective storage of digitalis bodies by the heart is not yet settled. While the values known for skeletal muscle have been obtained by perfusion experiments on inactive muscle, the values for the heart have been measured in preparations in which the heart is working. There is the possibility that the absence of muscular work may be a factor in determining the difference observed between the storage by the heart and the storage by striated muscle. In view of this, the influence of activity on the amount of k-strophanthoside taken up by skeletal muscle was analyzed. This was done by determining the *dosis certe lethalis* (d.c.l.) of said

glycoside for cats under dial anesthesia. In one half of the series of 80 cats the muscles innervated by both sciatic nerves were continuously stimulated at a frequency of 5/sec. The average d.c.l. for the cats whose muscles were thus stimulated was significantly higher than that for the control animals. This seems to indicate that the muscles in activity took up greater amounts of the glycoside. These results could have a bearing on the selective storage of digitalis bodies by the heart, which is in constant activity.

Differences in the effects of cold environment and of muscular work on adrenal function. A. DESMARAIS (introduced by L. P. DUGAL). *Depart. d'Acclimatation, Inst. de Biologie Humaine, Univ. Laval, Quebec, Canada.* It has been shown by Selye (*Arch. internat. de pharmacodyn. et de thérap.*, 55: 432, 1937) that adrenalectomized rats adapted to muscular work (revolving cages) are able to perform more work than non-trained, non-adrenalectomized rats. Since cold temperature is being regarded as an alarming stimulus having the same effects on adrenal function as muscular work, it was of interest to observe if adrenalectomized rats adapted to cold would resist better than non-adapted, non-adrenalectomized animals. The results show that the latter group, contrary to what one could expect, is significantly more resistant to cold than the adrenalectomized, adapted group. The difference between both types of stress is striking for the above experiment, but less so if one compared adrenalectomized animals adapted and non-adapted.

Further observations on the hypotensive effects of intravenous injections of hypertonic solutions. INGRITH J. DEYRUP (by invitation) and WILLIAM W. WALCOTT. *Dept. of Zoology, Barnard College, and of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* It was reported previously (Walcott and Deyrup. *Am. J. Physiol.*, 155: 1948) that, in the anesthetized dog, the intravenous injection of hypertonic solutions is followed by a fall in blood pressure which is consistently different from a marked and characteristic hypotension resulting from injection of mixtures of hypertonic solutions with homologous blood. Thus, whereas the injection of 12 to 30 ml. of 5 to 7 NaCl results in a diphasic fall in blood pressure, there is a profound and rapid single phase fall following injection of an equivalent volume of an equally concentrated mixture of blood and hypertonic NaCl. A related phenomenon has been observed in rabbits (nembutal, ether) but not in cats (nembutal). The striking difference between the cardiovascular response to injection of mixture of blood with hypertonic solutions, as compared with the effects of hypertonic solutions alone, suggests that the hypotension following the injection of such solutions may result, at least in part, from the interaction of some component of the blood with the hypertonic solution. In line with this hypothesis, we have obtained evidence that, under specifically defined conditions, a depressor substance may be demonstrated in approximately isotonic solutions derived from mixtures of hypertonic solutions with whole blood or erythrocytes suspended in 0.9% NaCl. An attempt has been made to characterize the depressor substance thus obtained, and to compare it with other depressor substances (various ions, histamine, compounds related to choline and adenosine).

Carbohydrate utilization and lactate formation in the chicken retina. H. F. DIERMEIER and V. F. LINDEMAN (introduced by Robert Gaunt). *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.* The retina has one of the highest rates of glycolysis of any tissue of the body. In order to find the source of the lactate produced by this tissue the following determinations were conducted on chicken retina kept at 37.5°C.: glycogen content before and after incubation for one hour; glucose utilization with varying concentrations of glucose in the incubation media; lactate production. Chemical assays were carried out by photometric analysis. All experiments were conducted under aerobic conditions. Ten experiments on matched retina are reported. Weights are given as wet weight. The average glycogen content (Morris, *Science* 107: 1948) immediately after removal from the eye was 0.0032 mg. glycogen (glucose equivalents) per mg. retina. After one hour of incubation in Krebs-Ringer solution with 0.1% glucose as substrate the value was decreased to 0.00227 mg/mg. of retina. The incubated retina consumed an average of 0.0205 mg. of glucose/mg. of tissue from the substrate and produced 0.00919 mg. of lactate/mg. of tissue.

In an attempt to account for the utilized carbohydrate not in the form of lactate, oxygen consumption measurements were made using the direct method of Warburg. The average oxygen consumption in the Kreb-Ringer solution with 0.1% glucose added was 0.507 cmm/mg. of tissue (wet wt.) per hour. Oxygen consumption with no glucose added as substrate was 0.279 cmm/mg. tissue (wet wt.) per hour. Oxygen consumption does not account for the remainder of the carbohydrate utilized and further experiments are in progress.

Identification of chloramphenicol (chloromycetin) degradation products by paper chromatography. WESLEY A. DILL (by invitation) and ANTHONY J. GLAZKO. *The Research Laby. of Parke, Davis and Company, Detroit, Mich.* The paper strip chromatographic methods of Consden, Gordon and Martin (*Biochem. J.* 38: 224, 1944) were applied to the separation of chloramphenicol (Parke, Davis) and its degradation products in urine. Using N-butanol with 2.5% phenol and 2% pyridine added, it was possible to separate chloramphenicol (I), a

hydrolysis product (II) and a conjugate (III) on a single strip of paper. After developing the chromatogram, colored derivatives were formed on the strip by reduction with titanous chloride, oxidation of excess reagent with bromine, diazotization with butyl nitrite and coupling with the Bratton-Marshall reagent. Small amounts of I and and II were found in the urine of man by chromatography after administration of chloramphenicol, with large amounts of III being present. Dog urine showed larger amounts of II than man. III was identified in the urine of man, dog, and rat, with the compound having approximately the same R_f value in all cases. The conjugate (III) was isolated by use of the counter-current extraction technic, and provisionally identified as the monoglucuronide. Following hydrolysis of III with β-glucuronidase, chloramphenicol (I) and free glucuronic acid were identified separately by paper chromatography. Two-dimensional paper chromatograms of urine were also prepared using N-butanol with 3% acetic acid and again with 3% ammonium hydroxide added. I, II and III were identified by comparison with known compounds, and, in addition, at least one other derivative of chloramphenicol was found to be present.

Potential gradient vs. current density in electrical control of axial polarity in regenerating *Dugesia tigrina* JEAN DIMMITT (introduced by GORDON MARSH). *Zoological Laby., State Univ. of Iowa, Iowa City, Ia.* Sections of *Dugesia tigrina* were exposed to direct current for 120 hours (original posterior end facing the cathode), in solutions of specific resistance from 478.4 to 52,157 ohm-cm., at 21°C. One hundred % mortality was found below 1000 and above 15,000 ohm-cm. For media of 1031 (previously reported), 2400, 7237, 10,605 and 12,600 ohm-cm., the potential gradients in mv/mm. across the regenerating pieces were constant for the following morphological effects, although the current densities varied 12-fold (values in parentheses are for 1031 ohm-cm., others are averages for all data): 178 (177) for regressive bipolars, in which head structures appear on both ends but disappear from the original posterior end within 3 days after removal from the current; 185 (193) for permanent bipolars; 198 (200) for progressive bipolars in which the head on the original anterior end is reorganized into a tail after removal from the current; and 217 (212) for complete reversal of axial polarity. Since constant current density is not the determinant of a given morphogenetic effect it may be concluded that control of polarity and differentiation is not determined by a stable relationship between the regenerating piece and its environment, nor by a critical rate of internal ion transport, i.e., by features of capacity. Constancy of potential gradient may be interpreted as the attainment of a critical discharge voltage (valence change) for some essential ion, orientation of molecules having a characteristic dipole moment, establishment of a liminal counter-E.M.F. or the overcoming of an inherent potential difference.

Antifibrillatory properties of N-methyl-N-(3,4 dimethoxybenzyl) β-(4 methoxyphenyl)-ethylamine hydrochloride. JOSEPH R. DIPALMA, JOSEPH J. LAMBERT (by invitation), RICHARD A. REISS (by invitation) and JOHN E. SCHULTS (by invitation). *Dept. of Physiology and Medicine, Long Island College of Medicine, Brooklyn, N. Y.* By use of a thyratron stimulator set at 600 impulses/min. and electrodes attached to the right auricle of intact open-chest cats the fibrillation threshold for the new compound was determined. Quinidine and α Fagarine were also assayed for comparative purposes. The new compound proved to be 2.3 times more active than quinidine and 1.3 times more active than α Fagarine. The average acute lethal i.v. toxicity for cats was less for the new compound as compared to quinidine and L Fagarine. On i.v. injection the new compound caused a transitory slowing of the pulse and a fall in blood pressure. In this respect and in the EKG changes produced with higher doses it resembled quinidine. It was also demonstrated that the refractory period of frog skeletal muscle was markedly prolonged by the new compound. In humans with normal hearts the new drug in equivalent doses i.m. caused a greater lengthening of the Q-T interval than did quinidine. Also in human chronic fibrillation the f-f interval was increased more by the new compound than by quinidine. The interesting features of the new compound may be listed as follows; it does not have a quinoline structure yet it is remarkably potent, its activity apparently stems in part from the proper substitution of methoxy groups, its structure is simple enough to offer important leads as to the basic chemical moiety necessary for antifibrillatory action and finally it may prove to be superior to quinidine and α Fagarine for the therapy of fibrillatory states in human beings.

Separate inhibition of resting and active oxygen consumption of functioning nerves. R. W. DOTY (by invitation) and R. W. GERARD. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* To study resting and active respiration, a modified capillary microrespirometer was developed to permit measurement of action potential and oxygen uptake of 10 separate nerves. The Q_{O_2} of nerves from one frog agree within 10%. The action of Na azide and methyl fluoroacetate (MFA) has been studied, yohimbin explored. Drugs were applied by soaking for an hour before mounting a nerve; stimulation was kept maximal at 120/sec.; temperature range was 20–25°C. Azide, 0.1 mM, and probably yohimbin, 0.01 mM, can depress the increased Q_{O_2} of activity while leaving the action potential height

normal. Resting Q_{O_2} may be somewhat depressed with azide, in which case conduction has gradually failed; but when normal, and with no increase on stimulation, conduction can continue unimpaired for at least 4 hours (confirming Bronk *et al.*). MFA, by contrast, is able to depress resting Q_{O_2} without diminishing the increase on stimulation. At 1 mM, resting Q_{O_2} is decreased 25% while the active increase is normal. At 5 mM, resting Q_{O_2} is halved and the active increase may be intact, depressed or even abolished. In any case, action potential may remain at normal height; one nerve, with the active increase present, being tetanized for 7 hours. In interpreting these results, the presence of respiring non-conducting elements in a nerve trunk must be considered.

'Saturation tension' and alveolar-arterial pO_2 gradient as expressions of 'venous admixture' in the lungs. J. C. Douglas (by invitation), R. L. Riley and A. Cournand. *Dept. of Physiology, Univ. of Western Ontario Medical School, London, Ontario, and of Medicine, Columbia Univ. College of Physicians and Surgeons, New York City.* The 'saturation tension' as measured with the Millikan oximeter during high oxygen breathing and the alveolar-arterial pO_2 gradient as measured by analysis of arterial blood and expired air during ambient air breathing have been studied in normal subjects and in patients with chronic pulmonary disease. Both methods provide information from which 'venous admixture' in the lungs can be calculated. The 'saturation tension' may be defined as the partial pressure of oxygen in the inspired air required to produce complete saturation of the mixed arterial blood. The required increase in pO_2 above atmospheric, in any given patient, is due chiefly to the lowering of the mixed arterial pO_2 by addition of blood which has been inadequately exposed to oxygen before entering the left heart. This blood has been called 'venous admixture.' The mixed arterial blood may be completely saturated in spite of a considerable proportion of 'venous admixture.' Increasing the inspired pO_2 raises the oxygen tension in poorly ventilated alveoli until at a certain inspired pO_2 all ventilated alveoli saturate the blood in contact with them. In addition blood perfusing non-ventilated portions of lung (or separated from alveolar gas by an impermeable barrier) is saturated by the excess dissolved oxygen in the blood from well ventilated areas. 'Venous admixture' as calculated from the alveolar-arterial pO_2 gradient during ambient air breathing involves the same factors but probably weights more heavily the effect of poorly ventilated alveoli.

Spectrophotometric determinations of Brilliant Vital Red and T-1824 in serum. Philip Dow and Raymond W. Pickering (by invitation). *Dept. of Physiology, Univ. of Georgia School of Medicine, Augusta, Ga.* Hamilton and collaborators (1932, 1940) have demonstrated that Brilliant Vital Red can be accurately and conveniently determined in the supernatant of an ethanol precipitation of serum by colorimetric comparison with similarly treated standards; T-1824 has universally been found to go down with the precipitate. Anomalies encountered in adapting the method for photoelectric colorimetry led to an investigation by Beckman spectrophotometry. As progressively more dilute ethanol (always 10 volumes) was used to clarify the serum (dog), a larger fraction of both dyes remained in solution, roughly paralleling the albumin solubility. The two dyes differed quantitatively, BVR being only 60% removed by absolute, not at all by less than 88% ethanol; T-1824, 93% removed by absolute, negligibly only at 80% ethanol. Below 82% ethanol, inconstant cloudiness vitiated determinations.

As previously emphasized, the ethanol precipitation provides a much clearer and more constant blank in the face of considerable variations in color and cloudiness of the original serum. Hemoglobin as such is removed completely. Some ethanol supernatants show no color whatever, even from severe hemolysis. In others an as yet unidentified derivative of hemoglobin (probably a ferriporphyrin) is extracted in small amounts. It absorbs comparatively little light at 508 or 625 mμ (the dye peaks) and has a high sharp peak at 404 mμ where the dyes absorb only slightly. Correction can thus be easily made by reading at two wave-lengths and applying the customary formula. Other methods of minimizing this contamination are being studied.

Cardiac changes in monkeys following explosive decompression to extreme altitudes. Roy Dowling (by invitation) and Samuel Gelfan. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Cardiac changes following explosive decompression to simulated altitudes of as high as 75,000 feet have been studied in anesthetized monkeys by means of unipolar lead electrocardiography, recording simultaneously from three extremity leads. Recompression of the animals at free fall rate began immediately after the sudden decompression. The first observable change in the e.c.g. complex is the displacement of the S-T segment, which occurs between 5 to 30 seconds after the sudden decompression. This change is quickly followed by an increase in the P-R interval and simultaneously an absolute shortening of the Q-T interval, even during the marked bradycardia that develops. The Q-T interval shortening indicates an acceleration of repolarization and the elevation of S-T segment in the VL and VF leads and depression in VR leads indicates that it is the left anterior ventricle that repolarizes faster than the rest of the heart. The increase in irritability of this part of the heart is also indi-

cated by the extra systoles that arise from the left anterior ventricle. Heart block ranging from first degree to complete block were also observed. The e.c.g. complex returns to normal after recompression to sea level.

These cardiac changes are due to the anoxia rather than the pressure changes. The same alterations have been observed in 100% N_2 anoxia without pressure changes. Similarly, sudden decompression from sea level to 40,000 feet in O_2, at which altitude the anoxia is insignificant, does not alter the e.c.g. complex. Roentgenograms show a dilation of the heart during the anoxia following decompression and also following N_2 anoxia.

Studies in pancreatic function. II. Statistical studies of secretin responses in individuals without pancreatic disease. DAVID A. DREILING (by invitation) and FRANKLIN HOLLANDER. *Gastroenterology Research Laby., The Mount Sinai Hospital, New York City.* Amylase, bicarbonate and volume responses to secretin were studied in 172 patients without pancreatic disease, to set up criteria of normalcy. One series of data, with Secretin-Wyeth alone, and another series with several different preparations were compared with data published by other investigators. Duodenal contents were collected by continuous suction through a double lumen gastroduodenal tube for 80 minutes following intravenous administration of 1.1 U of secretin/kg. body weight. This collection time was used, rather than the shorter intervals advocated by others, because it represents the entire period of secretory activity in response to hormone stimulation. Best agreement among the various series was shown by the means for maximum bicarbonate concentration in mEq/l.; also, the corresponding coefficients of variation were least. Poorest agreement and greatest scatter were shown by the amylase data, in activity U/cc. or total no. U/80 min/kg. Volume data in cc/80 min. or cc/80 min/kg. were intermediate. The following norms were established for future performance of this test with Secretin-Wyeth: maximum bicarbonate concentration, 92–124 mEq/l.; total volume, 2.0–4.4 cc/80 min./kg.; total amylase secretion, 8.0–20.4 U/80 min/kg. Bicarbonate and volume responses are more valuable than amylase determination, due partly to unknown pancreozymin content of different secretin preparations, and partly to alterations of vagal tone during the test. Nevertheless, amylase activity manifests sufficient statistical homogeneity to merit its inclusion in the study of pancreatic function.

Site of action of mercurial diuretics. JOHN J. DUGGAN (by invitation) and ROBERT F. PITTS. *Dept. of Physiology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* Two lines of evidence indicate that organic mercurial diuretics block distal tubular absorption of sodium. *1)* When saline is infused into normal dogs at such a rate that 800 to 1200 μEq. of sodium are excreted per min., the intravenous administration of 60 mg. Hg as mercuhydrin, increases sodium excretion to 2000 μEq./min. Doubling the dose to 120 mg. Hg increases sodium excretion to 2400 to 2500 μEq/min. Quadrupling the dose to 240 mg. does not increase it further. The administration of BAL returns excretion to the control range. It is apparent that mercuhydrin can block the absorption of a maximum of 1500 μEq. of sodium per min., a quantity roughly equal to 15% of that absorbed during the control periods and after BAL. This finding is consonant with the view that 80 to 85% of the sodium which is absorbed is absorbed in the proximal tubule (not mercurial-sensitive), and 15 to 20% in the distal tubule (mercurial-sensitive). *2)* The administration of 45 mg. Hg as salyrgan to a normal dog increases sodium excretion some 800 μEq/min. The infusion of 500 MU. of pitressin per hour increases excretion to a variable degree, i.e. from 200 to 500 μEq/min. The addition of salyrgan during pitressin naturesis increases sodium excretion only to the level attained with salyrgan alone. Apparently, pitressin and salyrgan block sodium absorption at the same site, presumably in the distal segment.

Effect of l-arterenol on the peripheral circulation in man. D. DUNCANSON (by invitation), T. STEWART (by invitation) and O. G. EDHOLM. *Univ. of Western Ontario, Ontario, Canada.* Forearm and hand blood flow were measured together with blood pressure and a continuous recording of heart rate with a cardiotachometer. L-Arterenol HCl. (noradrenaline) was injected intravenously with a constant infusion apparatus, at a rate of 2, 5 and 10 μg/min. for 5 minutes in each instance. The forearm blood flow showed no change with 2 μg/min. but with 5 or 10 μg/min. the blood flow showed a slight reduction in flow at a time when both systolic and diastolic blood pressure were raised. Hand blood flow showed a marked and consistent decrease with 5 or 10 μg/min. In most cases 2 μg/min. had no definite effect. The heart rate slowed consistently with infusions at the rate of 5 or 10 μg/min. In a few instances 2 μg. L-Arterenol also produced a definite slowing.

Relation of the adrenal and spleen in regulation of the leucocyte picture of the rat. ABRAHAM DURY and HABEEB BACCHUS (by invitation). *Dept. of Physiology, George Washington Univ. School of Medicine, Washington, D. C.* The relation of the adrenal and the spleen as a factor in the regulation of the total white cell count, and the absolute number of circulating lymphocytes, neutrophiles, and eosinophiles was investigated by determining whether statistical differences existed in groups of rats before and after adrenalectomy, spleenec-

tomy, adrenalectomy-spleenectomy, and sham operations as regards these leucocytes. A similar procedure was followed in these categories of operated groups of rats to determine the effect of adrenaline on the components of the leucocyte picture. It was found that the total white cell count was significantly increased only following spleenectomy or following adrenaline treatment of the adrenalectomized rat. None of the operative procedures altered the absolute number of lymphocytes; but these cells could be significantly depressed following adrenaline treatment only when both the adrenal and the spleen were in situ. The neutrophiles were significantly increased following either adrenalectomy or spleenectomy but were unaffected by adrenalectomy-spleenectomy. Following adrenaline treatment, the absolute number of neutrophiles was not significantly increased only in the spleenectomized group of rats. The number of eosinophiles was found to exhibit great variability from group to group of normal rats, but the evidence was clear that both the adrenal and the spleen were required if a significant depression in the circulating eosinophiles were to follow adrenaline treatment.

Arterial and venous potassium levels in man. D. P. Earle, S. J. Farber (by invitation) and J. D. Alexander (by invitation). *Dept. of Medicine, New York Univ. College of Medicine, New York City.* When proper precautions were observed the normal resting venous plasma potassium level in man was found to be approximately 0.5 mEq/l. less than generally reported. Blood was drawn into oiled syringes, transferred to tubes containing dried heparin and centrifuged immediately. Results were not affected by the use of a tourniquet to assist in obtaining blood, but muscular activity in the extremity raised the plasma K level. Hemolysis and allowing the cells to remain in contact with the plasma more than 10 minutes caused an increase in the plasma K level. At rest, arterial and venous plasma usually contain the same amount of K although at times there was more in the venous sample. Muscular activity raised the plasma K acutely and venous plasma from the extremity contained more K than the arterial. During intravenous infusions of glucose, venous plasma also contained more K than arterial, a difference which persisted following insulin in spite of a fall in both venous and arterial plasma K levels.

Measurement of cold and warmth thresholds of subjects exposed to environments from 18°C. to 38°C. Franklin G. Ebaugh, Jr. and Rudolf Thauer (introduced by Eugene F. Du Bois). *New York Hospital and the Depts. of Medicine and Physiology, Cornell Univ. Medical College, New York, N. Y.*. Using Hardy's radiation method, cold and warmth thresholds were measured on the forehead and trunk of subjects exposed to environmental temperatures from 15°C. to 38°C. Subjects were nude and to allow for adaptation remained for 2 to 3 hours at the same temperature and experimental conditions while measurements were made. The wall temperatures were not significantly different from the air temperatures. The cold thresholds remained at a mean value of 27 10^{-5} cal/sec/cm^2. in air temperatures of 15°C. to 25°C., but increased with the air temperature from 25°C. until at 38°C. the cold threshold (67 cal/sec/cm^2.) was more than twice that of the cooler environments. By contrast, the warmth thresholds remained at the mean value of 30 10^{-5} cal/sec/cm^2. throughout the range of air temperatures studied. This rise in the cold thresholds could not be explained by a change in the skin temperatures because at temperatures below 25°C. the cold threshold remained constant while the skin temperature gradually increased with the air temperature. However, the plot of room temperature against cold thresholds closely followed the curves of increasing peripheral blood flow with temperature published by Hardy and Soderstrom. Thus, it is possible that hyperemia of the skin increases the threshold of cold sensation but has no effect on the threshold of warmth sensation.

Effects of over and underperfusion upon coronary arterial blood flow. R. Eckel (by invitation), R. W. Eckstein, M. Stroud (by invitation), and W. H. Pritchard (by invitation). *Western Reserve Univ., Dept. of Medicine, Cleveland, Ohio.* If the size of the coronary vascular bed and its resistance to blood flow depends upon the oxygen supply and demand, it should be possible to show resistance changes when the vessels are perfused at various pressures with cardiac work constant. With a special perfusion apparatus the left descendens, or left circumflex or total left coronary arteries of anesthetized, heparinized, open-chest dogs were perfused with blood at various pressures which were suddenly changed. Aortic and perfusion pressures were measured with Gregg manometers and coronary inflow with a Shipley recording rotometer or a Gregg and Green differential flow manometer. In some cases, when the total left coronary artery was perfused, sinus outflow was also measured with a second rotometer. The resulting flows show a rapid marked increase in vascular resistance within a few seconds following perfusion at pressures above aortic. Likewise, when perfused at pressures below aortic, there is a marked sudden reduction in vascular resistance. Since these changes, although slightly delayed, occur also in coronary sinus outflow, it is believed that they represent automatic changes in vascular resistance which serve to meet the metabolic demands of the myocardium.

Response of coronary blood flow following stimulation of cardiac accelerator nerves. R. W. Eckstein, M. Stroud (by invitation), Charles V.

DOWLING (by invitation), ROBERT ECKEL (by invitation), and W. H. PRITCHARD (by invitation). *Western Reserve Univ., Dept. of Medicine, Cleveland, Ohio.* In the anesthetized, open chest and heparinized dog cardiac output, coronary artery inflow or coronary sinus outflow, and aortic blood pressure were measured before and after stimulation of the left cardiac accelerator nerves. The blood pressure was recorded with a Gregg optical manometer. The coronary flow was measured with a Shipley recording rotometer. Cardiac output was measured with a large Shipley recording rotometer placed in the thoracic aorta prior to ligation of the major proximal aortic branches. Cardiac output was varied and controlled by inflating a balloon in the left auricle. Control and experimental A-V differences between arterial and coronary sinus blood were determined.

Stimulation of the accelerator nerves produces an increase in vigor of contraction, cardiac output, cardiac work, coronary blood flow, and oxygen consumption. Simultaneous nerve stimulation and inflation of the left auricular balloon to reduce external cardiac work to below the control value is likewise followed by increased vigor of contraction, increased coronary flow, and increased oxygen consumption. Therefore it is suggested that the adrenaline-like substance released by nerve stimulation markedly increases myocardial O_2 consumption and reduces cardiac efficiency. Although direct effect of nerve stimulation upon coronary vessels must be considered, we have no evidence of increases in coronary flow in the absence of rising O_2 consumption.

Effects of x-radiation on water and electrolyte metabolism in the rat. ABRAHAM EDELMANN. *Biology Dept., Brookhaven National Laby., Upton, L. I., N. Y.* The intakes of distilled water, 1% NaCl solution, and food and the excretion of urinary Na, K, Cl and water by male albino rats were determined daily previous to and following exposures to 100, 250, 500, 800 and 1000 R of x-rays. Polydipsia and polyuria occurred during the first and fifth 24 hour period following radiation. The urinary excretions of Na, K, and Cl were increased at these times. Following doses of 500 R or more there is a relative and absolute decrease in intake of the salt solution. This is a monophasic response reaching its minimum at about 3 days. The salt solution intake returns to previous levels at 5 days. After doses of 100 or 250 R, the 1% NaCl solution intake follows the biphasic curve of water intake. Food intake decreased for 2 days following radiation and then returned to normal in about 2 days. Body weight changes paralleled the food consumption.

Responses to vagal stimulation during hyperthermia. H. E. EDERSTROM (introduced by A. B. Hertzman). *Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.* Dogs under anesthesia were heated to the lethal point by means of lamps. During the rise in body temperature the peripheral ends of the cut vagi were stimulated and electrocardiograms and optical recordings of blood pressure taken. The period of cardiac arrest due to vagal stimulation was reduced from several seconds at normal temperatures to a fraction of a second when rectal temperatures approached 45°C. The fall in systolic blood pressure induced by vagus stimulation was relatively smaller at high body temperatures. 'Vagus escape' was observed at all temperatures, but at 44–45°C. the rate of escape beats was about 3 times greater than those occurring during the same stimulation at normal body temperatures. Stronger stimuli gave similar results. In EKG tracings escape beats were characterized by absence of *P* waves when the right vagus was stimulated. Beats recorded during left vagus stimulation were more like the normal pattern. Bizarre and irregular *QRST* complexes were common in beats occurring during stimulation of the vagi at any temperature.

Effect of haemorrhage on forearm blood flow and heart rate in man. O. G. EDHOLM. *Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada.* Sixteen normal subjects were bled from 250–1300 cc. The withdrawn blood was retransfused approximately 4 hours later. Forearm blood flow was measured with a plethysmograph, and graphic records were obtained throughout the experiments with a cardiotachometer. During the period of haemorrhage, which lasted up to 30 minutes, there was no evidence of a diminished forearm blood flow except in one case whose control flows were high. In the 7 subjects who fainted, the usual vaso-dilatation occurred during the faint. Following the haemorrhage, in 9 non-fainters the forearm blood flow remained close to the control level for periods up to 4 hours. In 3 subjects, the blood flow diminished significantly below control values, but only during a period when the mean blood pressure was reduced. In the 6 subjects who fainted, the forearm blood flow remained at substantially control levels in 4 subjects. In 2 subjects the mean blood pressure was reduced throughout the period of the experiment and the forearm flow was likewise diminished. It was considered that these experiments indicated that the muscle blood vessels do not constrict to a significant extent after haemorrhage, and that there is no marked difference in this respect between fainters and non-fainters.

Effects of certain physical factors on the in vitro gastric secretion of the dog. CAROLYN TROWBRIDGE EDWARDS and LESLIE E. EDWARDS (introduced by ROBERT W. RAMSEY). *Dept. of Physiology and Pharmacology, Medical College of Virginia, Richmond, Va.* The method, previously reported, for *in vitro* gastric secretion has been improved and standardized, using the mucosa of the dog. Dissec-

tion techniques and a method for pressure control are described by which it is possible to obtain more consistent data. It has been shown that secretion can take place in one direction only as reversal of the membrane results in complete failure of secretion. This method has been used to show the effects of temperature on the secretion rate.

Acid secretion by gastric mucosa of the bull frog in vitro. LESLIE E. EDWARDS and CAROLYN TROWBRIDGE EDWARDS (introduced by ROBERT W. RAMSEY). *Dept. of Physiology, Medical College of Virginia, Richmond, Va.* Using the method for securing pure gastric secretion in vitro previously reported by the authors (*Federation* Proc., 1948), acid secretion by the mucosa of the bull frog (Rana catesbeiana) has been obtained. *p*H values (as low as 1.5), free acid and total acid concentrations and titration curves are comparable to those for mammalian gastric juice. After an initial delay of about 1 hour, secretion with a *p*H of less than 2 can be obtained continuously for approximately 3 hours. Subsequently the *p*H gradually rises while the volume of secretion increases. Total chloride determinations indicate the presence of neutral chlorides as well as HCl.

Comparison of intracardiac and intravascular temperatures with rectal temperature. LUDWIG W. EICHNA, ADOLPH R. BERGER and BERTHA RADER (introduced by DAVID P. EARLE, JR.). *Dept. of Medicine, New York Univ. College of Medicine, New York, N. Y.* In a study of the thermal gradients and deep tissue temperatures of 12 afebrile patients, thermocouples were introduced into the rectum, into the femoral artery and vein, and, by catheterization, into the right heart, pulmonary artery, superior and inferior venae cavae and the hepatic, subclavian and internal jugular veins. The data are presented as differences between the temperature of each intravascular site and the rectal temperature. Within the pulmonary artery, right ventricle, right atrium and femoral artery the temperatures usually were identical and averaged slightly less (0.2°C.) than the rectal temperature. This difference, though small, was consistent and in 10 of 12 comparisons the intravascular values fell below the rectal values by 0.13°C. to 0.34°C. Within the superior vena cava the temperature was lower than in the right heart, and in the subclavian vein its average value was 0.48°C. below rectal temperature. Within the inferior vena cava the temperatures were variable and their average approximated closely that of the right heart. The temperatures deep in the hepatic vein and high in the internal jugular vein were considerably above the values in the veins into which these vessels drain and these hepatic and internal jugular vein temperatures equalled the rectal temperature. The data demonstrate the thermal gradients within parts of the vascular tree, indicate sites (liver, brain) of increased heat formation at rest, and suggest that femoral arterial temperature is perhaps a more desirable index of 'mixed' deep tissue temperature than the usually accepted rectal temperature.

Photo-electric measurements of the arterial O_2 saturation dynamics in man for study of cardiopulmonary function. J. O. ELAM (by invitation), J. L. EHRENHAFT (by invitation), W. N. ELAM, JR. (by invitation), and H. L. WHITE. *Laby. of Applied Thoracic Physiology (Surgery) and Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.* Evidence favoring the use of histamine iontophoresis of the ear lobe for oximetry technique suggested a re-evaluation of the saturation responses currently employed in the assay of cardiopulmonary function. Observations were made with the ear spectrophotometer and several experimental oximeters on the histamine flushed ear lobe and the heated pinna of normal subjects. The lung-to-ear circulation time measured from the inspiration of helium to the initial saturation decrease averaged 4.1 seconds when the histaminized lobe was used and 5.2 seconds when the heated pinna was used. Ear-to-ear circulation time measured from the initial saturation decrease with a single inspiration of helium to the secondary decrease after recirculation averaged 21 seconds with the histaminized lobe and 24 seconds with the heated pinna or lobe. The rate of saturation increase when subjects changed from air to oxygen was found to be significantly greater when the histaminized lobe was employed. In these measurements the period required for maximal saturation increase (saturation time) ranged from 15 to 30 seconds when determined from the histaminized lobe depending upon the minute respiratory volume. Using the heated pinna the saturation times ranged from 73 to 148 seconds in agreement with the Millikan data reported by others. The histaminized lobe technique provided significant saturation deflections within 4 to 7 seconds when hyperventilation, breath holding, and single inspirations of oxygen or nitrogen (interspersed during quiet air breathing) were performed. The technique using the histaminized ear lobe affords information more representative of arterial dynamics than does the conventional Millikan procedure.

Blood coagulation studies in relation to menstruation and menorrhagia. RICHARD M. ELGHAMMER (by invitation), BURTON J. GROSSMAN (by invitation), PETER V. MOULDER (by invitation), ARTHUR K. KOFF (by invitation) and J. GARROTT ALLEN. *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* A series of 183 coagulation studies were made on 52 women during their menstrual cycles. Sixteen women were considered normal in reference to menstruation, and 36 suffered from menorrhagia without apparent cause. The studies included the whole blood clotting time, prothrombin time, protamine

titration, platelet count, and observation for gross evidence of lysis. In the menorrhagic group, during menstruation, 82% showed a mild to moderate thrombocytopenia (29% with less than 100,000/mm³), 35 of 36 showed a prolonged whole blood clotting time, and 35 of 36 had an increased protamine titration. The intermenstrual studies on these women showed thrombocytopenia in 46% (8% below 100,000), 15 of the 36 had a prolonged whole blood clotting time, and 14 of 36 had an increased protamine titration. All patients had prothrombin values of 80% or better except two who had 72% and 76%. During menstruation 46% of the normal controls showed a mild thrombocytopenia. Nine showed slightly prolonged clotting times, and 5 of 16 had increased protamine titrations. Between menstrual periods 21% of the controls had mild thrombocytopenia. Six of the 16 had increased whole blood clotting times and 5 had abnormal protamine titrations. Evaluation of the extent of bleeding was made from careful clinical histories. Menstrual pads were not collected and analyzed for hemoglobin content.

Blood changes associated with cold diuresis. JOHAN W. ELIOT, RICHARD A. BADER and DAVID E. BASS (introduced by H. S. BELDING). *Quartermaster Corps, Climatic Research Laby., Lawrence, Mass.* Blood changes associated with cold diuresis were studied in conjunction with experiments on the renal and hormonal mechanisms of cold diuresis. The order of successive 2½-hour exposures to 27°C. and to 15°C. was alternated between men and in each man from day to day. In 23 experiments plasma proteins rose an average of 0.5 gm/100 cc., and the hematocrit rose 2.9% in the cold; in 12 experiments the corrected erythrocyte sedimentation rate rose 76% in the cold. In the experiments in which a warm exposure followed the cold these values fell an average of 0.5 gm/100 cc., 1.8% and 41% respectively. In 18 experiments on 5 men, the average increase in urine output in the cold over output in the warm was 287 cc, which corresponded to an accompanying average plasma loss of 277 cc as as calculated from increases in plasma proteins and hematocrit and an estimated plasma volume of 50 cc/kg. However, a series of 15 experiments in which cold diuresis was largely or completely abolished by small doses of pitressin, but plasma proteins rose 0.4 gm/100 cc. and hematocrit rose 2.6%, indicates that there is no direct connection between changes in urine output and plasma volume in the cold. Further indication is supplied by considerable individual variations, well outside the error of the methods, between increased urine output and calculated plasma volume loss. Plasma chloride levels showed no significant change during cold diuresis, despite the fact that in 11 experiments average total chloride output in the cold was 2330 mg., compared to 1279 mg. in the warm.

Non-utilization of mannitol in uremic patients J. RUSSELL ELKINTON. *Dept. of Internal Medicine Yale Univ. School of Medicine, New Haven, Conn.* Although 100% recoveries of mannitol in urine have been reported (Elkinton, *J. Clin. Invest.* 26: 1088, 1947; Clark and Barker, *Proc. Soc. Exp. Biol. & Med.* 69: 152, 1948), evidence has been presented for utilization or clearance from plasma by non-renal excretory routes (Berger *et al.*, *Proc. Soc. Exp. Biol. & Med.* 66: 62, 1947; Dominguez *et al.*, *J. Lab. Clin. Med.* 32: 1192, 1947). In 4 uremic patients given 25.45 gm. of mannitol intravenously, little evidence of mannitol utilization was found for periods of 6 hours to 3 days. Mannitol utilized was calculated as the difference between the mannitol retained and the product of the conc. in serum water x the original mannitol space corrected for changes in chloride conc. and balance. In patients *2* and *4* (anuric) utilization was less than the analytical error, and in patients *1* and *3* was less than 12% of the non-renal excretory clearance rate of 21 cc/min. calculated by Dominguez *et al.* Masking of utilisation by an identical rise in serum blank, or failure of diseased kidneys to metabolize mannitol are alternative explanations.

PATIENT	MANNITOL				
	Time	Conc. Serum H_2O	Retained	Calc. in ECF	Utilized
	hr.	*mg %*	*gm.*	*gm.*	*gm.*
1	3	128.0			
	6.5	118.4	25.16	24.30	0.86
1	4	78.1			
	6.2	77.2	24.90	24.86	0.04
3	4	83.2			
	23	77.7	24.96	22.38	2.56
4	4	55.0			
	69	52.2	25.36	25.84	−0.48

Alterations in reflex muscular contractions induced by positive intrapulmonic pressure. LEONARD H. ELWELL (by invitation) and JOHN W. BEAN. *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* The knee-jerks of young adult men, elicited by constant stimulation at 4-second intervals by a Lombard hammer, were recorded kymographically, together with spirometer tracing, thoracic and abdominal movements, and changes in blood oxygenation as determined by oximeter applied to the ear. Positive intrapulmonic pressure (20 cm. H_2O) was applied through a rebreathing tank filled with room air and provided with soda lime canister and a pressure spirometer. Positive pressure was maintained for periods of about 3 to 7 minutes duration. Gentle application of pressure caused an immediate augmentation of the knee-jerk which persisted throughout the period of increased pressure; in several experiments the knee-jerk became

pendular in character. Respiratory minute volume was increased by a deepening and slowing of breathing; in some subjects frequency as well as depth was increased. The thorax was expanded in both its inspiratory and expiratory positions; in some cases the maximum inspiratory position was attained after several respiratory cycles rather than immediately. Thoracic respiratory movements were augmented. Changes in the abdominal region were in general similar to, but much less pronounced than, those of the thorax, except for a few cases where the abdominal circumference decreased rather than increased with positive pressure application. Changes in oximeter readings were so small as to be considered of little or no significance as causative factors in the reflex changes. All these effects were rapidly reversed on return to normal intrapulmonic pressure.

Separation of natural estrogens by countercurrent distribution. LEWIS L. ENGEL (by invitation), WILSON R. SLAUNWHITE, JR. (by invitation), PRISCILLA CARTER (by invitation) and IRA T. NATHANSON. *Medical Labys. of the Collis P. Huntington Memorial Hospital of Harvard Univ. at the Massachusetts General Hospital, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Separation of the naturally occurring estrogens and in particular the separation of estrone from estradiol are difficult procedures. The development of the countercurrent distribution technique for the separation of closely related substances promised to aid in the simplification of this problem. Solvent Systems consisting of ternary and quarternary mixtures were found in which a satisfactory spread of the partition coefficients of estrone, estradiol and estriol may be obtained. This makes possible a separation of the three urinary estrogens using a 24-plate distribution in the Craig countercurrent distribution apparatus. Other solvent systems were devised in which the partition coefficient of any one of the three compounds was nearly unity, thus placing the material in the optimum region for the detection of impurities. The partition coefficients of the three estrogens have been measured in the solvent systems employed in the isolation of the phenolic fraction from acid hydrolyzed urine. For these studies a fluorometric method based upon the procedures of Jailer and Bates has been used. With a Coleman Model 12-A Electronic Photofluorometer and a filter system consisting of a 436 mμ incident light filter and a 525 mμ interference filter for the emitted light, a full scale deflection of the instrument is obtained with 0.26 μg. of estrone, 0.36 μg. of estriol and 0.50 μg. of estradiol. The reaction is carried out by heating the estrogen in 1 ml. of 90% sulfuric acid and diluting with 65% sulfuric acid. An accuracy of ±10% is obtained.

The hepatic venous circulation with special reference to the sphincter mechanism. H. E. ESSEX and W. D. THOMAS (by invitation). *The Mayo Foundation, Rochester, Minn.* The existence of a sphincteric or throttle mechanism in the hepatic circulation of the dog has long been recognized. Mautner and Pick believed the mechanism resided in the smaller hepatic veins. Bauer, Dale et al held that it consisted of muscle rings in the hepatic veins near their confluence with the vena cava. The latter view has gained wide acceptance. A series of experiments made in this laboratory by Grana, Essex and Mann has shown that intact dogs are killed by intravenous doses of extracts of *Ascaris suum* that have little if any effect on a hepatectomized dog. It was also shown that the flow of blood through an isolated liver was arrested or markedly reduced by injections of such extracts into the portal vein. Casts made by injections of vinyl acetate into the hepatic blood vessels have shown that intravenous injections of extracts of Ascaris, histamine digitoxin, horse serum into sensitized animals (anaphylactic shock) and occlusion of the hepatic artery for two hours cause intense spasm of the hepatic veins giving a cork-screw or spiral appearance, particularly to the casts of the smaller vessels.

In a series of exploratory experiments similar spasm of the hepatic veins has been produced in some but not all the cats injected and also in white rats. The data are insufficient to warrant drawing a final conclusion concerning this question except in the dog. In contrast with what occurred in the hepatic venous system the portal venous system shows marked engorgement.

Cholinergic nature of the vestibular receptor mechanism: forced circling movements. C. F. ESSIG (by invitation), J. L. HAMPSON (by invitation), P. D. BALES (by invitation) and H. E. HIMWICH. *Medical Division, Army Chemical Center, Maryland.* Freedman and Himwich observed that the intracarotid injection of di-isopropyl fluorophosphate (DFP) produced righting movements in rabbits: the head turned away from the injected side and forced circling movements also in an adversive direction ensued while the homolateral pupil became miotic. Subsequent work revealed, that the intracarotid injection of appropriate doses of freshly prepared DFP in normal saline under local procaine anesthesia, produced similar results in other species: guinea pigs, dogs, cats, and monkeys. Preliminary experiments indicate that the internal ear must be intact for the altered head posture and forced circling to occur, for labyrinthectomized rabbits failed to show the characteristic response following the injection of DFP on the labyrinthectomized side. A rabbit with unilateral section of the eighth nerve and a similarly prepared cat failed to exhibit the characteristic responses to DFP. Extirpation of the cerebral cortex on the in-

jected side did not prevent the syndrome if the vestibular apparatus was intact. The mechanism of vestibular stimulation is indicated by the drug used to produce the turning seizures for DFP is a powerful anticholinesterase agent which facilitates the accumulation of acetylcholine. Atropine and scopolamine which shield the organism from an excess of acetylcholine corrected the abnormal posture and stopped the forced circling movements.

Experimental production of femoral fractures. F. GAYNOR EVANS, HERBERT E. PEDERSEN, JOHN F. HAYES, and HERBERT R. LISSNER (introduced by M. MASON GUEST). *Dept. of Anatomy and Engineering Mechanics, Wayne University, Detroit, Mich.* Sixteen experiments were performed on 13 femora of white males and 3 of Negro females ranging in age from 45 to 85 years. 'Stresscoat' deformation patterns, with a lacquer sensitivity of 0.00125 inches per inch, and fractures resulted from static torsion loading, loading of the greater trochanter and cross-bending loading of the center of the shaft. Torsion loading produced spiral fractures of the shaft; cross-bending loading gave transverse comminuted fractures of the shaft; loading of the greater trochanter resulted in intertrochanteric, transverse neck, subcapital and abduction fractures of the neck and trochanteric region. In trochanteric loading bones from females fractured at 548.3 lb. (average), males at 885.6 lb. (average). Elevating the trochanter increased the load supported without fracture because the effective bending force on the shaft is decreased and the direct compressive force increased. Under similar test conditions in our small series, there was an inverse relation between load producing failure and age of the individual from whom the bones were obtained. Loads producing failure with torsion and cross-bending loading were approximately equal in magnitude and considerably less than those producing fracture when the greater trochanter was loaded. All fractures closely resemble similar types seen clinically. 'Stresscoat' deformation patterns indicated that all types, with the possible exception of the rare abduction fracture, resulted from failure of the bone under tensile stress.

In vitro metabolism of bone marrow in Ringer-bicarbonate medium containing no glucose. JOHN D. EVANS and ROBERT M. BIRD (introduced by EUGENE F. DUBOIS). *Dept. of Physiology, Cornell Univ. Medical College, New York, N. Y.* The aerobic metabolism of rabbit bone marrow has been studied *in vitro* at *p*H 7.3 in Ringer-bicarbonate media containing no glucose. The rate of oxygen consumption under these conditions is 3.6 microliters/mg. of cell protein per hour. This is essentially the same rate as observed when glucose has been added to the system. Total aerobic acid production is small averaging 0.4 microliters/mg/hour. In the absence of glucose, small amounts of lactic acid appear to be consumed, averaging 1.0 micrograms/mg/hour. The R.Q. averages 0.84 as compared with one of 0.95 obtained in the same system to which glucose has been added. The amount of total reducing substances in marrow under our experimental conditions is small. Its utilization averages 1.1 micrograms/mg/hour. Analyses for total amino acids and non-protein nitrogen give no evidence for protein breakdown. From these data it appears clear that carbohydrate and protein breakdown are not sufficient to account for the oxygen consumption of marrow in a medium containing no glucose. It is inferred that the breakdown of fatty substances is primarily responsible for this oxidation.

Lymph flow during edema formation. GEORGE FAHR and HERMAN KOSCHNITZKE. *Minneapolis Gen. Hospital, Minneapolis, Minn.* The rate of lymph flow is a very important factor in reducing the rate of accumulation of edema fluid when ultrafiltration of plasma fluid into the interstitial spaces is accelerated. One of the three large lymph vessels near the ankle of a dog was cannulated and connected to the barrel of a tuberculin syringe before plasmapharesis. After plasmapharesis had been carried on for a period of 12 hours the colloid osmotic pressure was 5.8 mm. Hg. The colloid osmotic pressure of the lymph collected from the cannula was 3.7 mm. Hg. Before plasmapharesis it was only possible to get a lymph flow out of our cannula at the rate of 1 cc. in 9 minutes and 25 seconds when passive motion of the limb was carried out. Lymph flow after plasmapharesis was spontaneous and the rate was 1.5 cc. in 15 minutes. As there were 3 large lymph vessels the total flow of lymph from the foot of the dog was 0.3 cc. per minute. The weight of the foot from the point of cannulation down was 246 gm. or 1.2% of body weight. If we assume that the mean filtration pressure in the capillaries of the dog during these experiments was 22 mm. Hg. and at the same time assume that the tissue pressure under these conditions of edema formation was 5 mm. Hg. and then make use of Pappenheimer's filtration constant (Pappenheimer and Soto-Rivera, *Am. J. Physiol.* 152: 480, 1948) we calculate that .5 cc. of fluid would have been filtered per minute in the hind legs of a cat whereas we find .3 cc. lymph flow.

Resuscitation from fresh water drowning. DAVID C. FAINER (by invitation), CLEMENT G. MARTIN (by invitation), HENRY SCHWERMA, and A. C. IVY. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Studies on fresh water drowning have been made on dogs. The first stage lasting 68 seconds (mean), is characterized by struggling. The second stage, lasting 56 seconds (mean) is characterized by apnea or irregular respiratory movements. The third stage starting 124 seconds (mean) after submersion is ushered in by a precipitous fall in blood pressure which levels off

and reaches zero 259 seconds (mean) after submersion. No dogs survived after the precipitous drop in blood pressure occurred. Using an endpoint of 4 to 10 seconds after the cessation of struggling, no significant difference in survival was noted in dogs with a cannulated femoral artery if they received no resuscitation (survived 10, died 11), manual artificial respiration (survived 12, died 12) or artificial respiration with a mechanical alternating positive and negative pressure resuscitator (survived 12, died 8). In dogs without femoral artery cannulation, no significant difference in survival was noted if they received no resuscitation without postural drainage (survived 9, died 3), no resuscitation with postural drainage (survived 10, died 2) or artificial respiration with the alternating positive and negative pressure resuscitator (survived 4, died 2). Regardless of the measures employed, dogs having little or no staining of the lungs with methylene blue (added to the water used in drowning) survived, while those having dense staining died. About one-half of those dogs with moderate staining died. We interpret these results as indicating that mechanisms other than simple asphyxia play a role in drowning.

Oximeter measurements in pulmonary and cardiac disease. J. K. W. FERGUSON, D. M. FINLAYSON and I. M. HILLIARD. *Dept. of Pharmacology, Univ. of Toronto, and the Toronto Western Hospital, Toronto, Canada.* Three measurements were investigated: *1*) the difference in oximeter readings breathing air and breathing oxygen (A–O diff.); *2*) the time for the oximeter reading to change ½ way to its final reading on changing from oxygen to air (½ R time); *3*) the effect of exercise on the oximeter reading. The following groups of subjects were examined: *a*) 41 normals; *b*) 7 congenital heart cases; and then a group of miscellaneous pulmonary afflictions excluding tuberculosis and detectable cardiac disease, subdivided as follows: *c*) 20 lobectomies and pneumonectomies; *d*) 28 others showing no fall in O^2 saturation (less than 1%) on exercise; *e*) 27 others showing more than 1% fall in O_2 saturation on exercise. Group *c* included the most dyspneic. Out of 20 in group *c*, 12 had a fall in O_2 saturation on exercise. Average values for A–O diff. ± S.E. were as follows for each group: *a*) 4.0 ± 0.17; *b*) 4.9 ± 0.24; *c*) 3.17 ± 0.29; *d*) 3.86 ± 0.24; *e*) 4.94 ± 0.38. For ½ R time (in minutes) values were: *a*) 1.7 ± 0.07; *b*) 0.9 ± 0.04; *c*) 1.88 ± 0.22; *d*) 2.82 ± 0.28; *e*) 3.31 ± 0.23. Group *b* had the greatest fall in O_2 saturation on exercise (8–22%, av. 13.3%). Group *c* illustrates well the difference between pulmonary reserve and pulmonary 'efficiency.' Reserve is low, particularly in those who show a fall in O_2 saturation on exercise, while 'efficiency' is high in the same group, as shown by the low A–O diff. and short ½ R time.

Significance of activation of prothrombin in serum. JOHN H. FERGUSON. *Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N. C.* In spontaneous clotting of blood a variable and often considerable amount of prothrombin escapes conversion and persists, unlike the activated thrombin which is lost by the antithrombic mechanisms. In the presence of the suboptimal calcium content of serum, successive additions of optimal thromboplastin (brain extract) each activate some residual prothrombin to thrombin, which gradually disappears. The incomplete activations are proved by the repeated enhancement of coagulant activity, without exhausting the prothrombin even after 6 or more thromboplastin additions. However, if optimal Ca is added, complete exhaustion of prothrombin occurs after only 2 or 3 thromboplastin treatments. That more than one thromboplastin addition was found necessary suggests some 'antithromboplastic' activity of serum. The substrate for measuring thrombic activity in the above experiments) was tested prothrombin-free ($BaSO_4$-adsorbed fibrinogen. If a prothrombin-containing fibrinogen or plasma was used as substrate, an additional thrombin formation occurred which could be fully accounted for (as shown by control tests, minus serum) by transfer of Ca and thromboplastin in the serum activation mixture. Tests with suboptimal (1.2 mM) $CaCl_2$ were particularly significant since the extra thrombin from the substrate, in this case, was minimal. $BaSO_4$-adsorbed serum (prothrombin-free) yielded no thrombin on treatment with Ca and thromboplastin (tests on prothrombin-free fibrinogen). With its natural suboptimal Ca and optimal added thromboplastin, this serum had no influence on the weak thrombin formation in prothrombin-containing substrates.

Tissue respiration in the polar cod (*Boreogadus saida*) as a function of temperature. JOHN FIELD and C. N. PEISS (by invitation). *O. N. R. Arctic Research Laby., Point Barrow, Alaska and Dept. of Physiology, Stanford Univ., Calif.* The polar cod found off the north coast of Alaska is active at environmental temperatures as low as −1.5°C. At such temperatures many temperate zone poikilotherms are in cold narcosis. The present study was designed to throw some light on the metabolic factors which must underlie the capacity for functional activity at these very low temperatures. Brain and liver were removed from over 100 polar cod of similar body size by the moist cold box technique. Measurements of the Q_{O_2} of whole brain mince and of liver slices were made at 0°, 5°, 10°, 15°, 20° and 25°C. by the Warburg method. For each organ the log Q_{O_2}-Centigrade temperature curve was approximately rectilinear over the range tested. Moreover the slopes of the curves were nearly the same. Comparison of the log Q_{O_2}-Centigrade temperature curve for polar cod brain with a similar curve for black bass brain mince (*Huro*

salmoides, habitat temperature about 18°C.) established the following differences between these arctic and temperate zone forms. *1*) At each temperature the Q_{O_2} of polar cod brain was several times greater than that of black bass brain. *2*) The log Q_{O_2}-Centigrade temperature curve for bass brain showed a change in slope at 10°C., below this the slope was steeper (increase in Q_{10}); whereas the corresponding curve for polar cod had a constant slope from 0° to 25°C. Both these differences as exhibited in the polar cod brain curve are advantageous in arctic adaptation.

Blood production and iron reserves in man. STUART FINCH, DONALD HASKINS and CLEMENT A. FINCH (introduced by M. O. LEE). *Dept. of Medicine, Harvard Medical School, the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.* Five normal subjects were phlebotomized at weekly intervals. During 7 weeks of bleeding, between 1 and 1½ gm. of iron were removed from each subject. About 800 mg. of iron were mobilized for hemoglobin production during this time. Measurements were made of the rate of blood production and of the various hematological changes during the period of bleeding and the period of recovery. Available iron would appear to be the limiting factor in hematopoesis. Patients with large iron stores do not develop the degree of anemia shown by normal subjects. However, previous prolonged iron feeding in 2 of the subjects had no effect on their ability to tolerate phlebotomy. In those subjects who were allowed to spontaneously recover, serum iron values had not returned to normal in a period of 6 months. These studies provide information regarding the rate of hematopoesis in normal subjects under stress of phlebotomy, of the amount of available iron and of the time necessary to replenish the depleted iron stores.

An anti-coagulant present in testicular extracts. S. FISCH and E. J. TOWBIN (introduced by E. F. ADOLPH). *Dept. of Physiology, Univ. of Rochester, Rochester, N. Y.* Some crude hyaluronidase commercial preparations from bull testes were found to inhibit blood coagulation. However, the anti-coagulant is not hyaluronidase but a non-dialyzable material precipitated by various concentrations of ammonium sulfate. It is inactivated by heating at 65°C. for 10 minutes. The most potent preparation yet obtained, which has a very low hyaluronidase content, has an activity such that 5 mg. of the dialyzed and dried precipitate will produce a 13-fold increase in clotting time of 1 ml. of recalcified citrated human plasma or freshly drawn human blood. The anti-coagulant is not heparin, for titration with salmine over a wide range of concentrations has no effect. Moreover, the effects of these substances, in themselves anti-coagulants, are additive to those of the testicular substance. It does not act by binding calcium. It does not appear to be fibrinogenolytic nor fibrinolytic. It is neither anti-thrombic nor anti-prothrombic, these conclusions lending further support to the contention that it is not heparin. It appears to delay clotting by acting as an antithromboplastic agent. Only one other such agent has been described (Tocantins).

Electrophoretic study of the water soluble proteins of normal and atrophied muscles. E. FISCHER, N. J. COPENHAVER (by invitation), B. GALESKI (by invitation), and K. W. RYLAND (by invitation). *Baruch Center of Physical Medicine, Medical College of Virginia, Richmond, Va.* Electrophoresis of the water-soluble proteins of normal gastrocnemii (rabbit) reveals 5 main fractions, of which 4 could be tentatively identified as myogen (43.6%), myosin β (24.3%), myosin α (15.1%), and myoalbumin (9.6%). The migration velocity at *pH* 7.5 increases in the above order, and the small unidentified fraction has a velocity between that of myosin α and myoalbumin. During denervation atrophy, myoalbumin increases from the beginning, while myogen and the myosin fractions diminish considerably only after a delay of 10 to 12 days. After 3 weeks of denervation, the myogen fraction and the myosin fractions are nearly halved, while myoalbumin is increased three to four times. After tenotomy, myogen decreases and myoalbumin increases within 5 to 6 days as much as after 2 weeks of denervation. Simultaneous denervation and tenotomy changes the electrophoretic pattern considerably less than simple denervation or tenotomy. Enzymatic studies of the extracts confirmed that the combined operations damage the muscle less than the single operations.

Sensibility of man to light anoxia. P. E. FISET (by invitation) and L. P. DUGAL. *Dept. d'Acclimatation, Institut de Biologie Humaine, Univ. Laval, Québec, Canada.* Standard and objective psychological tests (concerning mechanical ability, space relations ability and survey of object visualization) were used in order to establish a difference in susceptibility to light anoxia (10,000 ft., 4 hours a week, 14 weeks) between 2 groups of human subjects receiving respectively, by demand valve system, oxygen and compressed air. The results show that the progress in performance from the 1st to the 2nd trial is significantly better for the group receiving oxygen with all the tests used. Confirmation using the same tests, and others, in the same conditions, has been obtained with 20 other human subjects.

Production of constrictive pericarditis in dogs. A. P. FISHMAN, L. H. RUBENSTEIN, L. W. SENNETT, K. KURAMOTO (introduced by L. N. KATZ). *Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago, Ill.* Incident to the investigation of the cardiodynamics in congestive heart failure in dogs, it became necessary to establish a consistently effective method for the production of

constrictive pericarditis. It was found that an envelope of polythene placed around the surface of the heart resulted in a rapidly progressive constrictive pericarditis with effusion. Within 2 weeks neither coronary vessels nor auriculoventricular grooves could be identified. The surface markings of the heart were completely obliterated. Within 4 to 6 weeks, distended neck veins, hydrothorax, pulmonary congestion, hepatomegaly, ascites and peripheral edema uniformly ensued. The predominance of any individual back-pressure manifestation varied with the site of intra-cardiac obstruction. The polythene did not enter into the inflammatory reaction and could be readily removed from between the thickened, scarred pericardial surfaces. If the polythene was not removed, the dogs died of constrictive pericarditis in 4 to 6 weeks. An attempt was also made to induce pericarditis in the intact animal. Thorotrast, injected under fluoroscopic vision, was used to outline the pericardial sac. Sodium morrhuate was then injected intra-pericardially. A sero-fibrinous pericarditis, without construction, resulted. The use of polythene provides a simple effective method for the production of a rapidly progressive constrictive pericarditis in dogs.

Enzymes in the grasshopper egg. LAURENCE ROCKWELL FITZGERALD (introduced by J. H. BODINE). *Zoological Labys., State Univ. of Iowa, Iowa City, Iowa.* The alkaline phosphatase of the developing egg of the grasshopper, *Melanoplus differentialis*, is shown to be apparently formed by the serosa and localised in the extra-embryonic fluid. In comparing this with other enzymes studied during the development of this same material, it is found that the enzymes of the grasshopper egg fall into 3 groups: *a*) Enzymes apparently of maternal origin, including methyl butyrase and tributyrinase. These enzymes decrease in amount (or total activity) during development. *b*) Enzymes which develop principally in the embryo. This group includes cytochrome oxidase and cholinesterase. *c*) Enzymes which appear during development, and which are found in extra-embryonic materials. This group includes alkaline phosphatase, tyrosinase, and catalase. The members of this last group are either undetectable, or barely detectable, in the embryo during development, but are swallowed, along with the extra-embryonic fluid just as the hatching process begins. The failure to detect these enzymes in the embryo does not necessarily mean that they play no part in development, as Haldane has shown, using data on saccharase, that a relatively small number of enzyme molecules may be sufficient for the normal functioning of a cell.

A small plastic tubing technique for right and left heart catheterization. H. F. FITZPATRICK, T. G. SCHNABEL, JR., and L. H. PETERSON (introduced by H. C. BAZETT). *Dept. of Physiology, Univ. of Pennsylvania Medical School, Philadelphia, Penna.* By the use of small plastic tubing, cardiac catheterization can be accomplished by a simplified procedure without skin incision and without apparent risk of venous thrombosis. In dogs, a routine venipuncture of the right external jugular is done with a special 18-gauge needle the lumen of which permits the passage of a small plastic catheter. The needle then is withdrawn. As the catheter is advanced slowly toward the heart, continuous ink recordings of venous pressure gradients can be obtained with a capacitance manometer. Without fluoroscopic guidance satisfactory measurements of right auricular and ventricular pressures may be made. From a manifold of 3-way stopcocks, blood samples for analyses can be withdrawn at a rate of 1 ml. or more per minute. Left ventricular pressures in dogs can be obtained by an open method, using a long, 18-gauge, olive tipped needle which is introduced, via the common carotid artery, to a level 1 inch above the aortic valve. Through this needle, a small plastic catheter is advanced beyond valve and into left ventricle. The needle is then withdrawn. The characteristics of this relatively short and very small plastic tubing permits reliable recordings of pressure waves which probably are not inferior to those obtainable by the much larger and longer catheters commonly used. This method of right heart catheterization is being evaluated on man with the purpose of determining cardiac output. Simultaneous electrocardiograms are made.

Thiocyanate and mannitol space and native ionic balance in acute adrenal insufficiency. JAMES B. FLANAGAN (by invitation) and R. R. OVERMAN. *Division of Physiology, Univ. of Tennessee College of Medicine, Memphis, Tenn.* It has been suggested that the urinary loss of Na and Cl in untreated adrenalectomized dogs is insufficient to account for the total reduction of these ions in extracellular fluid. Similarly, it has been proposed that urinary water loss in this condition is insufficient to explain the apparent extracellular dehydration. Simultaneous measurement of the volume of fluid available for the dilution of NaSCN and mannitol combined with flame photometric analyses of Na and K and chemical analysis of Cl in plasma and erythrocytes makes possible the calculation of the total amount of 'extracellular' Na and Cl in *1*) normal dogs, *2*) dogs in acute adrenal insufficiency, and *3*) adrenalectomized dogs maintained on DCA. Together with Na, Cl and water intake and output studies, it was calculated that on the average, the total 'extracellular' Na of adrenalectomized dogs maintained on DCA was 568 mM. The average total 'extracellular' Na of dogs in acute adrenal insufficiency was 347 mM. Thus the average total reduction in 'extracellular' Na was 221 mM. Since the dogs showed an

average negative Na balance (intake minus output) of but 50 mM, an average of 171 mM of Na remains unaccounted for. Similarly the total 'extracellular' Cl averaged 433 mM in dogs maintained on DCA and but 273 mM in dogs in acute insufficiency. The difference between these figures is 160 mM. However, the dogs showed an average negative Cl balance of but 79 mM, thus leaving 81 mM of Cl unaccounted for.

Persistence of hypertension after removal of the causative ischemic kidney. JACK FLASHER (by invitation) and DOUGLAS R. DRURY. *Dept. of Physiology, School of Medicine, Univ. of Southern California, Los Angeles, Calif.* Persistent hypertension develops in the rabbit as a result of unilateral renal ischemia. If this ischemic kidney is removed after the hypertension has lasted some time, the blood pressure does not necessarily revert to normal, but may continue above normal for several weeks. A control group of normal rabbits subjected to unilateral nephrectomy did not show any significant change in blood pressure after the operation. The findings support the view that a hypertension that has lasted some time tends to be self-perpetuating, persisting for an appreciable time after the original cause has been removed.

Comparison of heptazone (4,4-diphenyl-6-morpholinoheptanone-3-hydrochloride) with other analgesic drugs. LARS FLATAKER (by invitation) and CHARLES A. WINTER. *Merck Institute for Therapeutic Research, Rahway, N. J.* Comparisons have been made between heptazone, dl-methadone, dl-isomethadone, and morphine, for analgesic activity, toxicity, analgesic tolerance, and side effects. Heptazone, orally or parenterally, proved to be about twice as potent as methadone in rats, though of relatively short duration, but only half as potent as methadone in dogs. Isomethadone gave less consistent results than the other drugs. The outstanding advantage of heptazone was its low acute toxicity. L.D. 50 in mice (subcutaneously) averaged 210 mg/kg., compared with 33 mg/kg. for methadone. When dogs were dosed twice daily (heptazone 4 mg/kg., other drugs 2 mg/kg.) analgesic tolerance appeared in about a month. Side effects, such as narcosis, sedation and general depression, were most marked with methadone and morphine, much less with heptazone, and least with isomethadone. Nausea and vomiting occurred frequently in non-tolerant dogs given morphine, occasionally in methadone-treated dogs, but was never observed after heptazone or isomethadone. After analgesic tolerance was established, dose for all drugs was doubled. The increased dose was well tolerated by all dogs except those receiving methadone. Dosing was continued for 4 months, the drugs withdrawn for 1 month, then a second course of injections was given. After analgesic tolerance was established the second time, cross-tolerance studies were made. With the doses used, dogs tolerant to morphine were also tolerant to heptazone and isomethadone, but not to methadone. Those tolerant to methadone or isomethadone were tolerant to all the other drugs. Dogs tolerant to heptazone were tolerant to morphine and isomethadone, but only partially tolerant to methadone.

Fractional conditioning of behavior based on electrically induced convulsions. STEPHEN FLECK (by invitation) and W. HORSLEY GANTT. *Pavlovian Laby., Phipps Psychiatric Clinic, Johns Hopkins Univ., Baltimore, Md.* Three dogs were subjected to electric convulsions through 2 cephalic jellied plate electrods. Two auditory stimuli were given: one 15 seconds before shock (inhibitory), the other 5 seconds before shock (excitatory). Approximately 30 experiments/dog were made during 2 months. The dogs became increasingly resistant and negativistic to the experimental situation. Specifically there was fighting with other dogs, hostility towards the experimenters and increased motor activity outside the camera. Inside in connection with environmental stimuli the dogs manifested extreme motor activity with struggling in the hammock and running movements, at times indistinguishable from motor phenomena observed during and after convulsions. The animals disregarded injury and the movements seemed purposeless. Stimuli that brought on this behavior were *1*) the experimenter leaving the camera and *2*) the 'inhibitory' stimulus. The 'excitatory' stimulus caused no specific reaction except occasional orienting responses. The respiratory tracings often paralleled the motor excitement following stimuli *1*) and *2*) cited above, i.e. the tracings approached the pattern seen during convulsions. Occasionally jerking of the hind legs occurred after these stimuli. The negative reaction to the 'excitatory' stimulus with preservation of the reaction to the 'inhibitory' stimuli is evidence of amnesia for the 5-second preconvulsive period—comparable to the retrograde amnesia in patients after shock and after head injuries. The conditioning of certain motor phenomena with failure to condition the total convulsive behavior demonstrates fractional conditioning, rather than complete duplication of the UR by the cr.

Studies on colchicine derivatives: toxicity and antimitotic effect. W. FLEISCHMANN, A. GOLDIN (by invitation), B. GOLDBERG (by invitation), L. G. ORTEGA (by invitation), A. D. BERGNER (by invitation) and G. E. ULLYOT (by invitation). *Medical Division, Army Chemical Center, Maryland; Dept. of Preventive Medicine, Johns Hopkins Univ. School of Medicine; and the Smith, Kline and French Labys., Philadelphia, Penna.* Colchicine arrests mitosis in metaphase. In view of the great

toxicity of this compound it seemed of interest to investigate the antimitotic effect of some of its derivatives. Toxicity was determined by intraperitoneal administration. Mice were implanted subcutaneously with sarcoma 180. Tumors were 7 days old at the time the animals were sacrificed, 6 hours after injection of various compounds. Corneal mitoses were studied on flat preparations. Spermatogonia were studied on pre-fixed smears stained with acetic orcein. The following compounds were studied: Colchicine (*1*), trimethylcolchicinic acid methyl ether d-tartrate (*2*), colchiceine (*3*), trimethylcolchicinic acid (*4*), N-benzoyltrimethylcolchicinic acid methyl ether (*5*), N-benzoyltrimethylcolchicinic acid (*6*), and N-acetylcolchinol (*7*).

COMPOUND	LD_{50}	LOWEST DOSE (MG/KG) AFFECTING MITOSIS		
		Cornea	Spermatogonia	Sarcoma 180
	mg./kg.			
1	3.5	0.35	0.35	0.88
2	64.0	3.2	3.2	3.2
3	84.0	84.0	21.0	21.0
4	200.0	inactive	200.0	200.0
5	20.0	35.0	1.0	5.0
6	>700.0	inactive	inactive	inactive
7	56.0	28.0	14.0	—

The relationship between chemical structure, toxicity and antimitotic activity will be discussed.

Intravenous aminophylline and coronary blood oxygen. ELWOOD L. FOLTZ, ALAN J. RUBIN and WILLIAM A. STEIGER (introduced by CARL F. SCHMIDT). *Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, Penna.* In 10 normal intact dogs under morphine-nembutal anesthesia, coronary sinus catheterization was accomplished under fluoroscopic guidance (*Am. J. Physiol.* 152: 341, 1948). Simultaneous arterial and coronary venous blood samples were collected during control periods and after injection of either saline or aminophylline. Following intravenous aminophylline, coronary venous O_2 saturation fell from 30.2% to 17.7% (mean values) and the unsaturation (A–V difference) rose from 62.1% to 74.1%. Compared with results where saline was injected, these data showed significant differences. No marked change was noted in mean arterial blood pressure, but the mean pulse rate increased from 145 to 165 beats per minute. Electrocardiographic records revealed no significant change in pattern as a result of catheterization or experimental procedures. Since previous studies demonstrated that the myocardial stimulating effect of aminophylline increases cardiac output and cardiac work, it is suggested that aminophylline increases cardiac oxygen consumption more than the augmented coronary flow increases the delivery of oxygen.

Narcosis and refractory phase in certain cerebral mechanisms. A. FORBES, A. F. BATTISTA (by invitation), P. O. CHATFIELD (by invitation) and J. P. GARCÌA (by invitation). *Harvard Medical School, Boston, Mass.* Stimulation of the sciatic nerve in cats under deep barbiturate narcosis has previously been shown to evoke a primary electric response in the sensory leg area of the cortex and a secondary discharge, more widespread in the cortex, with latencies from 30 to 80 milliseconds. When a series of stimuli is applied at more than 4/second, each stimulus evokes a primary response, but only the first evokes the secondary discharge. A rest of nearly a second is needed before another stimulus can evoke the discharge. This behavior resembles the Wedensky inhibition in peripheral nerve, in which a partial block stops all but the first of a series of impulses so frequent that each impulse after the first falls in the relative refractory phase following its predecessor and is therefore subnormal.

Our experiments dealt with the question whether the failure of all stimuli after the first of a rapid series to evoke a secondary discharge is likewise due to subnormal impulses encountering a partial block in the cerebrum. Increasing the depth of narcosis greatly prolonged refractory phase in the mechanism of secondary discharge and decreased the stimulus frequency required to hold the discharge in abeyance. We found nothing inconsistent with the view that the effect is essentially a Wedensky inhibition. Depression of blood pressure, respiration and cortical waves was related to blood analysis of narcotic, and from the combination of these observations the rate of elimination of narcotic was estimated; this provided a measure of the depth of narcosis.

Comparison of pharmacological activities of several antihistaminic agents. ROBERT T. FORD, SHIRLEY L. MCHUGH, VIRGINIA M. O'ROURKE, DOROTHY J. NIXON and GERALD Q. O'NEILL (introduced by KARL H. BEYER). *Medical Research Division, Sharp and Dohme, Inc., Glenolden, Penna.* Tripelennamine hydrochloride, Pyranisamine Maleate, Antergan, Thenylpyramine hydrochloride, Prophenpyramine hydrochloride, R.P. 3277, and several other compounds have been evaluated on the basis of their local anesthetic, antihistaminic, antiacetylcholine, and antihyaluronidase activities. The antihistaminic tests include the antagonism of the effect of histamine against the isolated guinea pig ilium, the isolated guinea pig tracheal chain, and the histamine blood pressure depression in intact dogs. The local anesthetic measures include the rabbit cornea and the electrical stimulation of the isolated rat nerve-

diaphragm preparation. The hyaluronidase test was the inhibition in rabbits of the effect of hyaluronidase test was the inhibition in rabbits of the effect of hyaluronidase on the intradermal spread of India ink. The antiacetylcholine test was the antagonism of acetylcholine stimulation of the isolated rabbit ilium. The data suggest that of these measures, other than the strictly antihistamine tests, the inhibitory effect of these agents on the spreading induced by hyaluronidase may be a relatively important factor in anticipating clinical efficacy.

Indirect estimation of cardiac output in man from carbon dioxide exchange. CYRIL A. FORSSANDER (by invitation), HERBERT KRAMER (by invitation), C. J. MARTIN (by invitation), COLIN WHITE (by invitation) and H. C. BAZETT. *Dept. of Physiology, Univ. of Pennsylvania Medical School, Philadelphia, Penna.* Recent reports have demonstrated a wide range in arteriovenous differences in blood from various organs, with a consequent contrast between the compositions of superior and inferior caval blood. Any vaso-motor reflex, or change in mechanical conditions, is liable to alter the composition of mixed venous blood by changing the relative proportions of different streams; consequently in estimating venous alveolar air tensions it is probably important not to ask the subject either to make any effort or to change his respiratory pattern. Simple rebreathing of a high oxygen mixture, adopting these precautions, sometimes gives indications of a short plateau in CO_2 tension after some 20 seconds, but this is particularly difficult to demonstrate in shallow (and relatively rapid), also in deep (tho relatively slow) breathers. Attempts are being made to demonstrate plateaus in all types of subjects by reducing the volume of gas used for rebreathing to a minimum (in which Scholander's micro method for gas analysis aids) by utilizing a double bag system to accelerate mixing in the lungs (according to Gladstone's Principles), and thirdly by use of various experimental mixtures of oxygen and carbon dioxide. Cardiac outputs have been calculated from such estimates of virtual venous alveolar air, arterial alveolar air and carbon dioxide output. The values so obtained have usually been higher than those reported by users of the old acetylene method.

Electrolyte and protein changes during acute pneumococcal infection in rats. CHARLES L. FOX, JR. and GILBERT ASHWELL (introduced by ALFRED GILMAN). *Dept. of Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.* Metabolic balance studies of Na, Cl, K, N and H_2O were made on individual rats infected intraperitoneally with a minimal dose of virulent pneumococci to ascertain some of the chemical changes that occur in a typical bacterial infection, terminating in death in 4 to 5 days. Within the first 24 to 48 hours after infection, the excretion of Na, Cl and K increased 250 to 1000% above the pre-infection levels. On the 3rd and subsequent few days of survival, the Na and Cl excretion dropped to much below the pre-infection values, and occasionally to nil; in marked contrast, the greatly increased K excretion continued until death. There was a steady loss of body weight of from 12 to 19% and nitrogen excretion decreased progressively. The rats reduced their intake of food and water after the second day and urine output gradually diminished. All balances after infection became negative. The net loss of K was especially large and increased steadily until death occurred. It is of interest that in one instance of spontaneous recovery, these changes occurred initially but on the 4th day the weight loss ceased, K excretion approached zero and, as the K balance became positive, body weight returned to normal. Tissue analyses were made of samples taken from moribund rats at a time when other rats had already died. Tissues from uninfected rats carried on the same diet to control the metabolism studies were also analyzed. Muscle tissues from infected animals showed a marked elevation in K content; the intracellular compartment appeared to have increased and the extracellular components decreased.

Electrolyte, nitrogen and protein changes that follow massive removal of red cells and plasma protein. CHARLES L. FOX, JR. and BETTY B. FREEMAN (introduced by ALFRED GILMAN). *Dept. of Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.* In a study of the chemical sequelae of acute blood loss and hypoproteinemia in dogs, from $\frac{2}{3}$ to $\frac{4}{5}$ of the blood was removed in 3 hours and replaced with mixtures of salts and water. The concentrations and balances of H_2O, Na, Cl, K, nitrogen and protein were determined before and after the massive hemorrhage. A most noticeable result was a 50% decrease in plasma K concentration followed by excretion of considerable K in the urine during the first 24 hours after bleeding. One to two days later, K excretion became extremely low while nitrogen excretion, which had been subnormal at first, rose sharply. Thus the K/N ratio initially became very high (10 to 15/1), then became very low (0.8/1) and after a few days returned to normal (3–5/1). The Na and Cl values were high or normal depending upon the volume and composition of solution infused but equilibrium was achieved rapidly. The massive blood removal resulted in hematocrit values ranging around 10% and plasma proteins ranging around 1.3 gm/100 cc.; nevertheless the dogs survived, no edema resulted and both red cells and proteins regenerated. When K was in-

cluded in the replacement solution (5 mEq/l.) the fall in plasma K was only partly counteracted and the K excretion was unaltered; the added K, however, seemed to reduce both the tachycardia and the volume of infusion needed to prevent circulatory collapse and renal failure.

Growth and O_2 consumption, as affected by X-irradiation, using the bacterium, Serratia marcescens. ANN C. FOWLE (by invitation) and KENNETH C. FISHER. *Univ. of Toronto, Toronto, Canada.* The growth of the bacterium, *Serratia marcescens*, is accompanied by an increased rate of oxygen consumption, from which presumably it derives energy for growth. When the bacteria are subjected to X-irradiation various disturbances of growth and cell division are produced. It has been found that although the bacteria which grow and divide after irradiation do so at a rate considerably slower than normal, they do so without any appreciable interference with the amount of oxygen associated with growth. Thus it appears that the primary effect of X-irradiation does not involve the energy reactions specifically concerned with growth.

Factors affecting constancy of vasomotor response to repeated intra-arterial injections of mecholyl and of epinephrine. LEWIS FRANKLIN (L.I.M.R. Fellow) (by invitation), HOWARD WAYNE (by invitation), J. MAXWELL LITTLE and HAROLD D. GREEN. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.* In evaluating vasomotor responses induced reflexedly or directly by chemicals, in dogs anesthetized with sodium pentobarbital, we have compared these responses with responses to standardized quantities of mecholyl or epinephrine. Venous outflow was measured at 10 sec. intervals from the physiologically isolated hind leg, perfused from the dog's own arterial system. To determine the constancy of response 116 intra-arterial injections of 0.08 μg. of mecholyl were made in 9 experiments and 35 injections of 1.0 μg. of epinephrine in 6 experiments. In intact preparations the coefficient of variation (coef. var.) for the maximum change of flow per 10 sec. interval was 12–41% (av. 22.6%) for mecholyl and 7–53% (av. 28.5%) for epinephrine. Severance of both vagi and both carotid arteries or the sciatic and femoral nerves did not improve the coef. var. Expressing the response as percentage change increased the coef. var. to 29.4%; when expressed as total change in flow (cc. of blood flow gained or lost) the coef. var. was 27.0%. At constant control flows, the response to both drugs, expressed as maximum change of flow, decreased with decreasing perfusion pressure. At constant perfusion pressures, the response to mecholyl decreased but to epinephrine increased with increase in control flow. Enough data are not yet available in which results were obtained at constant control pressures and flows to draw definite conclusions but the coef. var. appears to be about 10%. Another cause of variability may be the development of refractoriness near the end of prolonged experiments.

Further lowering of glucose tolerance following an induced hypothyroid state in Yale rats. THOMAS F. FRAWLEY and CHARLES W. BISHOP (introduced by GEORGE F. KOEPH). *Dept. of Medicine, Univ. of Buffalo School of Medicine, and Medical Research Labs., Buffalo General Hospital, Buffalo, N. Y.* A significant percentage of Yale-strain rats have been shown to have an abnormally low glucose tolerance. Sayers and Sayers (*Am. J. Physiol.* 141: 466, 1944) postulated that this diminished tolerance might be due to an inherited hyperactivity of the adrenalmedullary system. Studies were undertaken to determine the influence of thyroidectomy or propylthiouracil on this impaired glucose tolerance. The goitrogens provide a more specific method for studying the influence of hypothyroidism on carbohydrate metabolism heretofore equivocal with thyroidectomy due to the high percentage of aberrant thyroid tissue. A 35% solution of glucose (350 mg/100 gm. body weight) was administered intraperitoneally and blood glucose determined at intervals over a 5-hour period. One group of 5 Yale rats weighing 200–400 gm. was thyroidectomized and to another similar group propylthiouracil (.010 gm/100 gm. body weight) was administered daily by stomach tube. Glucose tolerance was determined at weekly intervals over a 5-week period. The average values (mg.%) for each group are shown in the following table:

HOURS	0	½	1	2	4	5
(T) Before	90	326	228	134	104	97
(T) After 5 weeks	89	395	420	295	200	164
(P) Before	80	280	243	154	102	119
(P) After 5 weeks	80	354	310	228	136	114

(T) = Thyroidectomy group.
(P) = Propylthiouracil group.

Quantitative measurement, by external counting, of the I^{131} content of the thyroid gland in man. A. STONE FREEDBERG, ALVIN URELES and MARVIN VAN DILLA (introduced by HERRMAN L. BLUMGART). *Med. Res. Lab., Beth Israel Hospital; Dept. of Medicine, Harvard Medical School; and Dept. of Physics, Mass. Inst. of Technology, Boston, Mass.* We have recently described a method for quantitative measurement of I^{131} content of the thyroid gland in man by external counting. Independence of size and location of the thyroid gland (depth geometry and absorption) was achieved by con-

necting in parallel 4–6 platinum cathode Geiger-Mueller counters, arranged in a horizontal plane in circles of varying radii. With these counters in a circle 36–120 cm. in diameter, a central source of radiation was moved about within a sphere 8–32 cm. in diameter concentric with the circle of counters; the maximum variation in counting rate was 5%. When the source was surrounded by muscle, bone, water and saline in quantities approximating the human neck there was no significant change in counting rate. With a constant amount of radioactivity in volumes varying from 5–350 cc. in different shaped containers no significant change in counting rate was observed. Identical amounts of I^{131} in 5 cc. and in 2,000 cc. of water placed in gallon jugs showed a decrease of 5% in the counting rate in the latter instance.

Preliminary studies on thyroid uptake in patients with varying gland size are consistent with mock up experiments. With a circle 52 cm. in radius 10 μc. in the thyroid gland gives a counting rate 2.5 background. Following a standard oral dose of 150 μc., I^{131} carrier-free, the thyroid uptake and urinary I^{131} excretion was studied in 22 subjects. Thirteen thyrotoxic patients demonstrated, at varying times up to 48 hours, a maximum uptake in the thyroid gland from 46–84% and averaged 66. In 7 euthyroid subjects the uptake ranged from 13–39% and averaged 30. In 2 myxedema patients the thyroid uptake was 1 and 4%. Preliminary studies on the rate of uptake in the thyroid gland have also been carried out and are described.

Effect of Eck fistula formation and meat intoxication on serum phosphatase and dye clearance of adult dogs. SMITH FREEMAN. *Dept. of Exptl. Medicine, Northwestern Univ. Medical School, Chicago, Ill.* The average preoperative Rose-Bengal dye-clearance of 19 adult dogs was 107 units and their serum phosphatase averaged 2.4 Bodansky Units. The minimum dye clearance after Eck fistula formation averaged 53 units and the corresponding phosphatase values averaged 9.9 units. Meat intoxication was produced in 10 of 11 dogs of the preceding series. The average serum values prior to meat intoxication were dye clearance of 51 units and phosphatase of 6.6 units. A diet of raw horse meat (50 gm/kg.) produced meat intoxication in an average of 9 days. The average dye clearance decreased to 34 units and the serum phosphatase increased to 18.8 units. These changes are construed as evidence of further liver injury in the Eck fistula dog.

Summation of stimuli studied with the aid of anticholinesterases. JEANE SISKEL FREY (by invitation) and ROBERT GESELL. *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* The carotid nerve was stimulated repetitively at frequencies ranging from 10 to 50/sec. and the effects of this stimulation on breathing recorded with the aid of a Hutchinson spirometer. Progressive increase of frequency of stimulation was attended by an increase of temporal summation of stimuli as shown by the increased respiratory response. Similar gradation of frequency of stimulation following administration of anticholinesterases (physostigmine, DFP, X substance (Army Chemical Center) and CO_2) was attended by an appreciably greater summation of stimuli at all frequencies. Potentiation of temporal summation by anticholinesterases agrees with an acetylcholine sparing action at synapses and a consequent intensification of neurocellular current which are believed to generate nerve impulses (current report of Hunter, Lillie and Gesell). Since anticholinesterases impair conduction of impulses in nerve fibers (Gilman, Nachmansohn, and Lowe and Gesell) it is unlikely that they potentiate reflexes by improved conduction of nerve impulses in the dendrites and cell body. For the reason that these reagents potentiate rather than depress central nervous activity, our experiments support earlier conclusions of Lowe and Gesell that impairment of impulse conduction in nerve and muscle by anticholinesterases is incompatible with the theory of synaptic conduction of nerve impulses. It is suggested that our experiments agree with the concept of specialization of function in the neuron.

Can transfer rate be calculated from urine isotope ratio? HERBERT D. FRIEDLANDER (by invitation) and WALTER S. WILDE. *Physiology Dept., Tulane Univ. School of Medicine, New Orleans, La.* The expression for calculating transfer rate is $P^*/P = \Delta n_1^*/\Delta n_1 = \Delta n_2^*/\Delta n_2 = \ldots = \Delta n_e^*/\Delta n_e$. P^*/P is the instantaneous ratio for tagged and for total but exchangeable chemical in the plasma. $\Delta n^*/\Delta n$ is the ratio of the respective chemicals, as infinitesimals, accumulated in the organs 1, 2, etc., or in an excreted fluid e, urine, saliva, etc. The amount of exchange back and forth of intrinsic substance, Δn, cannot be measured directly in most systems. It must be calculated from the equation. Certain excreted fluids such as urine, however, represent a continuous one-way sampling of Δn. Thus we measure Δn^* and Δn directly in urine collected over the transfer period and substitute them in the equation to calculate Δn_1 in an organ. This obviates estimation of P^* as $\overline{P}^*$ from the area under a plasma time-concentration curve for tracer.

$\overline{P}^*/P$ and $\Delta n^*/\Delta n$ were compared in 16 rats after subcutaneous tracer phosphate. Before 35 minutes $\overline{P}^*/P$ exceeds $\Delta n^*/\Delta n$. Non-tracer phosphate lying in the urinary channels at injection time probably contaminates Δn. Later $\Delta n^*/\Delta n$ for the full period exceeds $\overline{P}^*/P$ often threefold even at 150 minutes. Govaerts (*Arch. Int. Pharmacodyn.*

75: 261) presents evidence for non-exchangeable dog plasma phosphate. Calculations from his data agree with ours. $\overline{P}^*/P$ in plasma acid filtrates of the rat equals that in magnesia precipitates prepared in the cold. Faster urination of phosphate during the plasma peak hardly explains the result. Radiocalcium data sent by Govaerts yield similar $\overline{P}^*/P$ and $\Delta n^*/\Delta n$ values.

Inactivation or removal of insulin by the liver. A. FRIEDMAN (by invitation), H. F. WEISBERG (by invitation) and R. LEVINE. *Dept. of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* Under normal circumstances insulin, after leaving the pancreas, reaches the liver before it goes into the general circulation. We wished to determine whether any inactivation or removal of the hormone occurred during its passage through the liver. For this purpose we have injected insulin into dogs at a constant rate I.V. (0.1 μ/kg/hr.) over 2-hour periods, by two injection routes—the splenic vein and the femoral vein. Blood sugar values were estimated at 15–30 minute intervals. Each dog served as its own control. The results show that insulin infused via the splenic vein had, in all cases, a significantly smaller effect on the arterial blood sugar levels than did the insulin infused via the femoral vein. It is estimated from the areas of the curves that the liver led to removal or inactivation of about 40% of the injected insulin. These experiments were made with Lilly insulin which contains variable amounts of a hyperglycemic factor which might have been the cause of the lessened effect of intrasplenic insulin. However, identical experiments with a brand of insulin (NOVO) which we found not to have the hyperglycemic factor, also gave the lessened intrasplenic response.

Gastrointestinal motor effects of intestinal extracts. M. H. F. FRIEDMAN. *Dept. of Physiology, Jefferson Medical College, Philadelphia, Penna.* The experiments were performed on dogs under sodium pentobarbital, ether, or urethane anesthesia. Motor activities were recorded by means of tandum balloons and water manometers from the antrum, duodenum, terminal ileum, and colon. All tissue extracts were administered by vein. Certain preparations of intestinal mucosa, in doses of 2 to 10 mg/kg., always increased the tonus and force of contractions. This effect apparently occurred thruout the whole gastrointestinal tract but not in other smooth muscles. The response was obtained within a latent period of 2 minutes and was sustained for 10 to 45 minutes. Some evidence of tachyphylaxis was obtained. The motor effects were not abolished by bilateral vagotomy, splanchnectomy, separation of the intestine into segments, or large doses of atropine. No synergism between effective extracts and pilocarpine or mecholyl was noted. The motor effects were not due to histamine and probably not to choline. The effective principle was not found in extracts of gastric mucosa, or in other types of intestinal mucosa extracts. It could be concentrated from intestinal extracts made by the method of Greengard et al. for the preparation of enterogastrone, and was found present in a commercial preparation of "purified enterogastrone" which is available for the treatment of peptic ulcer. The results suggest, but do not prove, the presence of a gastrointestinal motor 'hormone' in the intestinal mucosa.

Rôle of the adrenal cortex in the excretion of purines. MEYER FRIEDMAN, DONALD BERNSTEIN (by invitation), and SANFORD O. BYERS (by invitation). *Harold Brunn Inst. for Cardiovascular Research, Mount Zion Hospital, San Francisco, Calif.* Twenty-eight rats (maintained on water containing sucrose 5%) were given 100 U/kg. of an extract of adrenal cortex (Lipo-Adrenal Cortex-Upjohn) by intramuscular injection. It was found that the total renal excretion of purine increased approximately 25% during the first 24 hours following the above injection. Six rats, injected with DOCA (5 mg/kg.), showed no appreciable increase in purine excretion during the next 24 hours. Twelve rats were nephrectomized and given the same amount of Lipo-Adrenal Cortex. Four nephrectomized rats were given DOCA. The total purine content of the blood of these 16 animals 24 hours after injection was no greater than that of 14 control nephrectomized rats. These observations suggest that Lipo-Adrenal Cortex effects in rats an increase in the excretion of purines which is of renal origin.

Emotional state on acquired and inborn reactions: satiation on cardiac conditional reflexes and unconditional reflexes to food. W. HORSLEY GANTT. *Pavlovian Laby., Phipps Psychiatric Clinic, Johns Hopkins Univ., Baltimore, Md.* In the laboratory the conditional reflexes maintain a mechanical constancy comparable to the physiological reflexes so that they can be expressed even in a formula. Outside the laboratory the reactions are characterized by extreme variability and apparent lawlessness. The explanation is to be found chiefly in the strict control of the state of the animal used in the laboratory. Our previous investigations showed that the salivary conditional reflexes (*crs*) were dependent upon the state of hunger of the animal, falling from a maximum to zero immediately after satiation. The cardiac *cr* has been measured in this laboratory for many years to study the inner emotional state of the animal contrasted with the more specific external *crs*. Several dogs with well established food *crs* were allowed to eat to satiation after taking control salivary and cardiac measures to the condi-

tional signals for food. An immediate decrease of the cardiac *cr* resulted from the satiation. The cardiac component of the unconditional reflex (*UR*) was only slightly affected (the animal would not eat after satiation unless the food were forced into the mouth). Satiation caused an elevation of control cardiac rate. For example before satiation 'Sechs' control heart rate = 85, cr = 110, UR = 110; after satiation control heart rate = 100, cr = 108, UR = 105: in 'Peik' before satiation control heart rate = 65, cr = 98, UR = 95; after satiation control heart rate = 85, cr = 95, UR = 110. The cardiac component of the inhibitory *crs* was not affected. It is thus evident that not only the specific salivary *crs* but the more generalized emotional *cr* components, represented by the heart rate, may be immediately abolished by a change of emotional state of the animal while the *URs* are are much less affected.

Anoxia on conditional reflexes in dogs. W. HORSLEY GANTT, GEORGE W. THORN and C. DORRANCE. *Pavlovian Laby., Phipps Psychiatric Clinic and Dept. Medicine, Johns Hopkins Univ., Baltimore, Md.* Changes in higher cerebral function caused by single exposures to moderate altitudes (18,000 ft.) are difficult to measure because of inadequate testing methods. Two dogs aged 11 and 4 years were exposed 4 hrs. to both 18,000 and 25,000 ft., either by reducing pressure or reducing oxygen content. One dog was exposed in a pressure chamber 6 times, the other 5 times. Responses measured were based on food ('Sechs') or pain ('Connie'). Sechs at 18,000 ft. showed a reduction of the conditional reflex (*cr*) from 238 U. control in the pressure chamber to 170 experimental; inhibitory *cr* remained 0. At 25,000 ft. the disturbance was more severe, excitatory *cr* = 150 U., inhibitory = 135. After 24-hr. recovery impairment persisted, excitatory *cr* = 205, inhibitory = 48 U. Connie having *crs* to painful stimulus, gave as control 83% correct motor responses to excitatory *cr*, 3% to inhibitory *cr*. At 18,000 ft. no impairment; at 25,000 ft. excitatory *cr* = 90% correct but inhibitory *cr* = 60%—disturbed differentiation. After 24 hr. there was complete recovery. Although differences according to age, temperament and kind of reflex measured, in both dogs there was marked impairment at 25,000 ft., but in the younger dog not at 18,000 ft. *Cr* measurements can be useful in evaluating efficacy of therapy for altitude tolerance. The *cr* impairment appears when there is no other observable neurological change.

Spinal cord pathways and connections for sensory fibers from the knee joint of the cat. ERNEST GARDNER (introduced by M. MASON GUEST). *Dept. of Anatomy, Wayne Univ. College of Medicine, Detroit, Mich.* Recordings were made from the dorsal surface and ventral roots of the spinal cord following electrical stimulation of the posterior nerve from the knee joint of the cat. With single shocks, potential changes characteristic of internuncial activity were recorded from approximately the 3rd lumbar to the 1st or 2nd sacral segments. These potentials were maximal in the 6th and 7th lumbar levels. Reflex discharges were recorded from ventral roots of those segments in which considerable internuncial activity was observed. Recordings were also made of action potentials in the posterior nerve conducted antidromically after single shocks to dorsal funiculi at various levels. Results indicate that articular fibers, after entering the spinal cord, synapse with internuncial neurons in several cord segments. These neurons in turn relay to motor neurons and also to lateral spinothalamic tracts. In addition, collaterals from the larger of the entering fibers ascend in dorsal funiculi as far as the medulla oblongata, beyond which they were not traced. In the lumbar cord, the fastest conduction rates were 90–100 m/sec. At the cervical cord, however, conduction was from 35–50 m/sec. The endings from which these ascending fibers are derived are Ruffini-type endings in the capsule of the posterior part of the joint.

Physical and chemical properties of emulsifying agents in urine. JOSEPHINE B. GARST (by invitation) and HARRY B. FRIEDGOOD. *Dept. of Medicine, Univ. of Calif. at Los Angeles; Calif. Inst. for Cancer Research, Los Angeles, Calif.* The urinary material which stabilizes oil-in-water emulsions encountered in extraction of urine with organic solvents has been separated by full saturation with ammonium sulfate at *p*H 5. The flocculent brown precipitate which forms can be separated more rapidly by addition of ether and shaking. The precipitate is separated from the urine and reprecipitated twice from distilled water by addition of acetone to 80% concentration. It is then washed at the centrifuge with small amounts of water. When centrifugation no longer sediments the material, it is precipitated finally with acetone. The precipitate is then dried in vacuo over sulfuric acid. The product is hydrophilic, has a total nitrogen content of 10%, gives positive Molisch, xanthoproteic and biuret tests. A solution of the separated precipitate in distilled water stabilizes an ether-in-water emulsion. On the other hand, the residual urine at *p*H 5, freed of the above material, does not form emulsions with ether. Electrophoresis of the product has been conducted at *p*H 8.6 in 0.1 molar barbiturate buffer. The soluble fraction consisted of two components in the ratio of approximately 3 to 1. The characteristics of the material suggest that it consists predominately of glyco-protein.

Electrocorticograms of the cytoarchitectural

areas of macaca mulatta. JOHN S. GARVIN and L. V. AMADOR (introduced by PERCIVAL BAILEY). *Dept. of Neurology and Neurological Surgery, Univ. of Illinois College of Medicine, Illinois Neuropsychiatric Institute, Chicago, Ill.* Investigators have noticed differences in electrical activity from various cytoarchitectural areas during other electroencephalographic experiments. Specific studies have been made on rabbits and cats. The cortex of *Macaca mulatta* was exposed and direct recordings made in anesthetized, unanesthetized and curarized animals. Electrical activity from various areas revealed marked differences. There were minor variations between animals, but in general the following holds. The tracings from areas *FA* (Area 4) and *PC* (Area 1) are quite distinctive, but indistinguishable from each other. The entire occipital region is lower in amplitude than any other region. *OC*, *OB* and *OA* (Areas 17, 18, 19) are practically alike except that the amplitude of *OA* is slightly higher. Activity is similar throughout all of *TA* (Area 22), and is not characteristic. The activity of *FB* (Area 6) is of high amplitude and resembles the activity from the inferior parietal region, while the activity from *FD* (Areas 10, 9) and *FD delta* (Area 46) is of rather low amplitude with some faster superimposed activity. Tracings from some areas, notably *PEm* (Area 5), *PE* (Area 7), *PF* (Area 40) and *PG* (Area 39) were indistinguishable, but were characteristic for those regions. Multiple simultaneous recordings within *FA* revealed 'Dial bursts' at one point and not at another and at times almost synchronous bursts throughout the area. No particular difference between the unanesthetized, curarized and anesthetized monkeys was observed except that the frequency in *FA* and *PC* appeared faster under curarization and without anesthesia.

Action of D O C A and adrenal cortical extract on body water and kidney function. MARIO GAUDINO and MARVIN F. LEVITT (introduced by HOMER W. SMITH). *Dept. of Physiology, New York Univ. College of Medicine, New York City.* Three normal dogs were injected with 30 mg. of D O C A for 3 weeks and the changes in extracellular space (inulin vol. of distribution), total body water (D_2O), intracellular space (difference between D_2O and inulin vol.), plasma vol. (T-1824) and renal function (inulin and PAH clearances, Tm_{PAH}) were studied frequently. There was a marked increase in the extracellular space with no change or slight decrease in the total body water, resulting in a significant decrease in the intracellular space. Plasma volume slightly increased or showed no change. Inulin clearance increased, PAH clearance increased very slightly and Tm_{PAH} was significantly decreased. The first change to appear was the increase in extracellular space. A fairly good correlation exists between the inulin clearance and the inulin space. With the prolongation of treatment the above changes tended to disappear, despite an increase in the dose of D O C A. After the injections were discontinued the return to normal was complete. Two dogs treated with 15 cc. of total adrenal cortical extract (Upjohn) revealed a completely different effect: the extracellular space did not change significantly, but the total body water increased markedly, reflecting an absolute increase in the intracellular space. Plasma volume, inulin and PAH clearances did not change. Tm_{PAH} increased in one case and decreased in another. All changes were reversed after the treatment was discontinued.

Arterial oxygen saturation and intracardiac pressures during acceleration in relation to cardiac damage. O. GAUER (by invitation), J. P. HENRY, E. E. MARTIN (by invitation), P. J. MAHER (by invitation). *Aero Medical Lab., Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio.* A fall in arterial oxygen saturation has been predicted during acceleration in view of the marked disturbances in lung circulation observed in X-Ray motion pictures. Therefore, in both human and animal experiments oxygen saturation in arterial blood has been continuously recorded during acceleration. A Kramer glass cuvette oximeter with an automatic syringe has been employed together with Van Slyke determinations of blood oxygen content. A fall from 97% to 90% in oxygen saturation was measured in a human exposed to 4.5 *g* positive acceleration for 15 seconds. Experiments in anesthetized dogs submitted to both positive and negative acceleration showed changes of the same order during brief acceleration and even greater changes during prolonged runs (2 min.). Intracardiac pressures were simultaneously recorded in both ventricles of nembutalized dogs during negative acceleration, using modified pressure sensitive tip Wetterer catheters. Carotid and jugular pressures were also recorded in these experiments. These measurements were an attempt to correlate the above mentioned fall in arterial saturation with the frequent development of subendocardial ecchymoses in the left and right ventricles during negative acceleration. During the acceleration there were changes in the contour of the right intraventricular pressure wave suggesting that at certain periods during the cardiac cycle the changes in right intraventricular pressure may fall out of phase with those occurring in the left ventricle. Such pressure differentials between the heart chambers during acceleration indicate a possible mode of origin of the above mentioned ecchymoses.

Utilization of fructose by the perfused brain of the living cat. A. GEIGER, J. MAGNES, R. M.

TAYLOR and H. WAELSCH (introduced by H. GRUNDFEST). *Depts. of Biochemistry and Psychiatry of New York State Psychiatric Inst., New York City.* Fructose penetrates into the narcotised cat's brain up to concentrations of 60–100 mg/100 gm., if the fructose concentration of the blood is kept very high (1.5–2%) for an hour or more. When the normal blood circulation of the cat's brain was switched over to perfusion with a glucose-free suspension of erythrocytes in a Ringer solution containing 100 mg. of fructose and 7 gm. bovine serum albumin/100 cc. the following was observed: The oxygen consumption of the brain was about half as great as with the same perfusion fluid containing glucose. The glucose from the brain disappeared at a rate corresponding roughly to the rate of oxygen consumption. As long as glucose was present in the brain the fructose concentration in it remained unchanged. Only after the complete disappearance of glucose did the fructose concentration slowly diminish. The amount of oxygen consumed during this period was larger than that required for the oxidation of fructose. The larger part of the oxygen consumed during the glucose-free period could be as preliminary experiments indicate, accounted for by the disappearance of glutamin and glutamic acid from the brain cortex.

Differences in cortically and subcortically induced convulsions. ERNST GELLHORN. *Lab. of Neurophysiology, Dept. of Physiology Univ. of Minnesota, Minneapolis, Minn. Cortically induced convulsions.* Convulsive activity induced by topical application of strychnine does not interfere with the independence and asynchrony of different cortical areas either under control conditions or in asphyxia. *Subcortically induced convulsions.* First, strychnine is injected into either hypothalamus (mammillary body) or thalamus (dorsomedian or ventrolateral thalamic nucleus) in 'Dial' cats and local spikes are recorded. Hereafter asphyxia is initiated which causes the spikes to disappear first and the normal potentials later. During this time the asynchrony existing between various cortical and subcortical areas persists unchanged. After cortical potentials have disappeared a synchronous outburst is recorded in cortex, thalamus, basal nuclei and hypothalamus and lasts for 10 to 30 seconds. This discharge consists of convulsive spikes and is accompanied by overt convulsions in the non-curarized cat. The experiments suggest that the subcortical focus does not control cortical activity as long as the cortical excitability remains normal. If, however, cortical potentials disappear the subcortical focus seems to be released (removal of cortical inhibition) and leads to a generalized synchronous discharge. *Systemic convulsions.* If generalized convulsive activity is induced by intravenous injection of picrotoxin asphyxia leads to synchronous cortical and subcortical discharges as observed in experimental *group 2.*

A skin resistance recorder or sudomotometer. WILLIAM A. GEOHEGAN (introduced by Joseph C. Hinsey). *Dept. of Anatomy, Cornell Univ. Medical College, New York City.* Measurement of skin resistance provides a convenient index of sudomotor activity, and recording of skin resistance changes is of great value in determining nervous pathways. While in some cases an approximate measure of skin resistance is desirable, in others, it is important to record small changes due to an applied stimulus. An instrument has been developed which permits measurements of both kinds, is simple to operate and is safe for application to human subjects in the operating room. A set of interlocking push buttons permits connection of any one of 5 electrodes to a bridge and vacuum tube voltmeter which is calibrated directly in ohms. The voltmeter output is connected through a stable d.c. amplifier to an Esterline Angus recorder. Sensitivity and stability are adequate for recording changes as small as 1%, and permit detecting presence of relatively few fibers in a stimulated pathway. A convenient balancing arrangement is provided to center the recorder for any resistance value from 1000 ohms to 100 megohms. The instrument is operated from the 115 volt a.c. line and employs no batteries. Electrical and mechanical isolation of the input circuit insure against accidental application of high voltage to the patient.

Cholinesterase activity of intact nerve; DFP inhibition. R. W. GERARD, B. LIBET and D. CAVANAUGH. *Dept. of Physiology, Univ. of Chicago and Marine Biological Lab., Woods Hole, Mass.* The inertness of ACh applied to nerve has been attributed to lack of penetration. Also, an objection raised to the measured ChE inhibition, on grinding a nerve soaked in DFP, has been that the inhibitor and enzyme react after structural breakdown. The following experiments invalidate these points. A frog sciatic or a single squid giant axone is threaded through a capillary, with cut ends outside. The capillary is filled (about 20 cu. mm.) with a dilute ACh solution, or (squid axone) a measured drop of the solution is moved to and fro along the nerve. After desired intervals at room temperature the ACh solution is removed and ACh assayed. With unpoisoned nerve or axone, 65 to over 80% of the ACh present (0.1 to 1.28) had disappeared in 20 minutes. Controls show that no ChE leaves the tissue, so ACh must have reached the enzyme in intact fibers. Despite adverse conditions (low ACh concentration, long diffusion distances, part time contact) this corresponds to a QChE of over one-fourth the full value of a squid axone. When

10 mM DFP was present in the ACh solution, no ACh loss was found in 60 minutes with frog nerve. Conduction remained normal.

Species reactivity of staphylocoagulase and staphylokinase. EARL B. GERHEIM (by invitation) and JOHN H. FERGUSON. *Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N. C.* A study was made of sheep, cattle, rat, dog, horse, guinea pig, chicken and rabbit plasmas in connection with the staphylococcal factors concerned in coagulation (prostaphylocoagulase) and lytic activity (staphylokinase). The lengthened staphylocoagulation times, as well as the complete incoagulability of some plasmas was due to: *1*) an inhibitor to the active clotting agent, staphylocoagulase; *2*) destruction of fibrinogen by proteolytic activity due to activation of a proenzyme by the factor staphylokinase; and *3*) a possible low co-factor content. Fractionation of the plasmas with ammonium sulfate indicated that the inhibitor was quite ubiquitous, with the globulin fractions containing the most. No fraction of any species except rabbit contained as much cofactor as could be obtained from human plasma or serum. The fractionation procedure seemed to inactivate a great deal of the inhibitor. Parallel tests for fibrinolysis with staphylokinase and streptokinase were made. Staphylokinase, which has previously been shown to activate a proenzyme in human serum, was reactive with a proenzyme found in rabbit, guinea pig and dog serum. Streptokinase did not activate any proenzyme of the species tested, except human. Staphylokinase differs from streptokinase in requiring a long incubation period for maximal lytic activity. The greatest proteolytic activity was developed in dog serum.

Biophysics of the cochlea. B. E. GERNANDT (by invitation), J. S. RIESCO-MCCLURE (by invitation) and H. DAVIS. *Central Inst. for the Deaf, St. Louis, Mo.* Aural microphonics were recorded simultaneously from the round window of guinea pigs and from another electrode placed in a very small hole drilled into the scala vestibuli or media of the apex or one of the intermediate turns. Pure tones of various frequencies were adjusted in intensity to give a standard response of 20 microvolts at the round window. The necessary sound intensity was noted, and the corresponding voltages of the microphonic at the other electrode were measured. In several experiments the cochlea was then injured through another small hole at some intermediate position and measurements repeated. The logarithms of responses at apex (with round window response constant) plotted as a function of log frequency yield a smooth curve that is nearly horizontal below 1000 cps and falls off more or less rapidly as frequency is increased. Apical and round-window responses are usually equal at about 3000 to 4000 cps. Intermediate positions of the electrode yield similar curves with less low-tone emphasis. Injury abruptly increases the intensity required to produce a standard response to the frequencies that correspond in position to the position of injury. The relations of position of electrodes and of injuries to frequency correlate well with those previously established by Stevens, Davis and Lurie. Even a severe injury, however, rarely raises the necessary intensity more than 20 db. The significance of these and related observations to the biophysics of the cochlea are discussed.

Fractionation of enterogastrone concentrates with N-butyl alcohol. LEON L. GERSHBEIN (by invitation), HUGH M. MILLEN (by invitation) and A. C. IVY. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* A number of enterogastrone concentrates purified by way of the tannate (ET) as well as by subjecting the latter to picric acid treatment (EP), were submitted to alcohol fractionation. The extraction of these products with successive portions of aqueous alcohol and absolute methyl alcohol led to high recoveries of the starting materials with little enrichment of the hormone, as based on the continuous histamine assay method. However, at least a two-fold enrichment occurred with n-butyl alcohol. In fact, 50 mg. of one concentrate which gave a gastric stimulatory response in excess of 80% yielded a butanol extract eliciting 43% and 52% inhibition with 25 mg. doses. The yields of these fractions aggregated 13–14%. The procedure employed is exemplified by the following run: A mixture of 2.00 gm. of ET-16 and 200 ml. of butyl alcohol was mechanically stirred for 20 minutes at 32°–38°C. After filtration by centrifugation, the supernatant liquid was concentrated to 16 ml. and treated with 110 ml. of acetone. The precipitate which was washed by centrifugation with portions of acetone, then 1:1 acetone-ether and finally ether, weighed 268 mg. (13.4% recovery). Further fractionation of the butyl alcohol-soluble product was afforded by treatment with methyl alcohol. The 22% soluble portion was highly stimulatory to the dog, whereas the methylalcohol-insoluble fraction in a 25 mg. dose inhibited gastric secretion to the extent of 70% in the same dog.

Heating of tissues by near and far infrared radiation. JEROME W. GERSTEN (by invitation), KHALIL G. WAKIM, RICHARD W. STOW (by invitation) and ADRIEN N. PORTER (by invitation). *Sections on Physical Medicine and Physiology, Mayo Foundation and Mayo Clinic, Rochester, Minn.* It has been widely accepted that near-infrared radiation is more efficient in heating human tissues than is far-infrared. Because the evidence does not seem convincing a comparative

study of the heating effects of irradiation with energy in the far-infrared and near-infrared ranges was made. Tissue temperatures were recorded by means of copper-constantan thermocouples, and the radiant energy density at the skin surface was determined by means of a radiation thermocouple. Near-infrared rays were produced by a 250-watt mazda bulb, and far-infrared rays by a 450-watt carborundum heater. The energy absorption/u. volume of tissue was calculated at the end of one minute of heating. With both sources of heat, the skin absorbed the greatest amount of energy, and the muscle the least, as computed/u. volume of tissue. When the radiant flux density at the skin surface was almost identical, the energy absorption by the tissues was 22% greater with the carborundum unit than with the mazda bulb. The distribution of the energy absorption was the same with both sources, thus indicating that the effectiveness of the carborundum heater was not due to marked cutaneous heating with relatively little deep heating. When the output of the carborundum unit was decreased by 43.5%, the energy absorption by the tissues was decreased 40.8%, thus indicating that when the same heater is used the energy absorbed is directly proportional to the energy radiated. Judged by the increase in temperature of tissues to a depth of 15 mm., this study showed that far-infrared radiation is more efficient than near-infrared radiation.

Tissue distribution, excretion and metabolic fate of chloramphenicol (chloromycetin). ANTHONY J. GLAZKO, LORETTA M. WOLF (by invitation) and WESLEY A. DILL (by invitation). *Research Labs. of Parke, Davis and Company, Detroit, Michigan.* Chloramphenicol is an aromatic nitro compound for which chemical and microbiological assay procedures have been developed. The chemical method is based on reduction of the nitro group to a primary amine with metallic zinc or titanous chloride, followed by diazotization and coupling. It is suitable for determination of total drug in biological material, since it includes inactive degradation products as well as unchanged chloramphenicol. The drug is rapidly absorbed, with serum levels reaching a maximum in about 2 hours and falling gradually during the next 18 to 24 hours. Analysis of tissues in the rat and dog reveal high concentrations in the kidney and liver, with progressively lower concentrations in the lung, spleen, heart, muscle and brain. Unchanged chloramphenicol has been isolated from the urine of man and identified. About 5% of the administered dose is recovered in 24-hour urine specimens by microbiological assay, whereas 80 to 92% is recovered by chemical assay, indicating the presence of large amounts of inactive nitro compounds. A simple hydrolysis product of chloramphenicol and a conjugate have been identified in urine. The conjugate was found to regain antibiotic activity following hydrolysis with a glucuronidase preparation. No increase in aryl amine excretion was observed in the urine of man or dog, but lower animals showed a significant rise. Incubation of chloramphenicol with rat liver slices *in vitro* resulted in loss of activity, with the formation of inactive nitro compounds and a small amount of aryl amine.

Site of action of soy bean trypsin inhibitor on the coagulation system of blood. MARY BETH GLENDENING and ERNEST W. PAGE. *Dept. of Obstetrics and Gynecology, Univ. of California Medical School, San Francisco, Calif.* Soy bean trypsin inhibitor (SBTI) markedly inhibits the rate of blood coagulation, and also protects animals against the toxic effects of intravenously administered thromboplastin. The findings of Tagnon (1946) and Macfarlane (1947) that the site of action is on some factor involved in the conversion of prothrombin to thrombin have been confirmed. The effect on thrombin is negligible. Initial experiments with plasma collected in silicone-coated apparatus indicated that the SBTI affected a plasma constituent as well as thromboplastin. A 2-stage technic with purified prothrombin as substrate and dilute beef serum as a source of accelerator globulin was utilized in order to identify this constituent. Thrombin formation ceases at approximately the same time regardless of the presence or absence of SBTI. The amount of thrombin formed, however, varies directly with the concentration of beef serum and inversely with the concentration of SBTI. Thromboplastin, freed of Ac-globulin is inhibited similarly but the amount of thrombin formed is much lower than when beef serum is present. In the absence of beef serum the phospholipid extracted from purified thromboplastin will not convert prothrombin to thrombin, although it becomes increasingly active with rising concentrations of beef serum. This suggests that an active constituent of beef serum, presumably Ac-globulin, can qualitatively substitute for a protein portion of thromboplastin. Present evidence supports the concept that both a phospholipid and a protein are essential for any appreciable conversion of prothrombin to thrombin, and that SBTI acts on both the protein moiety of the thromboplastin molecule and the serum Ac-globulin.

Effect of pteroylglutamic acid and 4-amino pteroylglutamic acid on the growth of sarcoma 180. A. GOLDIN (by invitation), B. GOLDBERG (by invitation), L. G. ORTEGA (by invitation) and E. B. SCHOENBACH. *Biology Sect., Medical Div., Army Chemical Center, Md., and the Dept. of Preventive Medicine, The Johns Hopkins Univ. School of Medicine, Baltimore, Md.* The ability of folic acid (pteroylglutamic acid) to reverse the effect

of aminopterin (4-amino-pteroylglutamic acid) induced inhibition of the growth of sarcoma 180 was tested. Groups of mice bearing transplanted sarcoma 180 were treated with aminopterin plus a series of concentrations of folic acid. The weights of the tumors were compared with the tumors of mice which received aminopterin alone, folic acid alone, and with the tumors of control groups. A partial reversal of the aminopterin induced inhibition of tumor growth by administration of folic acid was a constant finding. The tumors of aminopterin treated mice averaged 35% of the controls. The tumors of mice treated with aminopterin, plus folic acid ranging in concentrations from 5–50 mg/kg., were 65–75% of the weight of the controls. Folic acid itself appeared to produce some inhibition of tumor growth when administered at higher concentrations. The reversal of aminopterin inhibition by folic acid is reflected in the histological appearance of the tumors. Tumors of mice treated with folic acid did not show the marked histological changes resulting from aminopterin inhibition, but approximated that of the control tumors.

The reflexology of the pudendal nerve field. CHARLES H. GOOD (introduced by BERRY CAMPBELL). *Dept. of Anatomy, Univ. of Minnesota, Minneapolis, Minn.* Previous work on the reflexology of the spinal cord has dealt principally with the locomotor apparatus—the lumbo-sacral intumescence and the neuro-muscular apparatus of the hind limbs. Noteworthy are the fast components of the reflex in these locomotor fields and the absence of direct transmission of activity across the midline of the cord. The pudendal nerve mediates the sexual reflexes and other reflexes of the perineal-anal region. The effector structures are near the midline and frequently work in conjunction with their antimeres. Central latency is long, crossed return is the rule. Stimulation of the penis of cats results in an efferent discharge, crossed and uncrossed along the S_2 and S_3 ventral roots. The total latency to S_2 ventral root is 8.2 msecs. of which 2.2 msecs. is afferent conduction time. Crossed intersegmental as well as crossed segmental return is found. The forms of the reflexes are to be shown as well as their conditioned activity. Parallel anatomical study of the fiber spectrum of the pudendal nerve and its roots is being made.

Myocardial lactate and pyruvate metabolism in dogs under severe stress. WALTER T. GOODALE and DONALD B. HACKEL (introduced by W. FLEISCHMANN). *Physiology Sect., Medical Div., Army Chemical Center, Maryland.* Development in this laboratory of a technique of coronary sinus catheterization (*Am. J. Physiol.* 152: 340, 1948) has made possible the study of myocardial metabolism in intact nembutalized and unanesthetized dogs. Removal of metabolites from coronary blood was assumed to represent myocardial utilization, which was then calculated from the product of two apparently independent variables: *1*) Coronary blood flow, measured by the nitrous oxide method, and *2*) Coronary arterio-venous difference, estimated from simultaneous femoral arterial and coronary venous blood samples. Severe stress from adrenalin infusion, shock, hemorrhage, severe anoxia, convulsions, and severe nutritional deficiency all caused a rise in arterial lactate levels above normal, but removal of lactate from coronary blood by the myocardium was greatly reduced, occasionally even to zero at arterial lactate levels of 40 to 60 mg.%. Pyruvate removal was only moderately reduced. Although decreased lactate removal may indicate decreased myocardial lactate utilization, adrenalin release may be the common denominator in such situations of severe stress, leading to glycogenolysis within the heart (and liver), with endogenous production of lactate as an immediate emergency source of energy; with high tissue lactate levels produced endogenously, the myocardium should remove less lactate from coronary blood as the blood-to-tissue concentration gradient falls. Failure of the myocardium to remove lactate from coronary blood at elevated arterial lactate blood levels is apparently associated with a grave cardiac emergency, and often with a threat to survival.

Reduced synthesis of phosphocreatine in tissue homogenates from alloxan diabetic rats. E. S. GORANSON (introduced by R. E. HAIST). *Dept. of Physiology, Univ. of Toronto, Toronto, Canada.* The phosphorylation of creatine, under aerobic conditions, has been studied in homogenates of various tissues of the rat, principally brain, incubated for short periods at 37°C. In the presence of added succinate, cytochrome C and adenosine triphosphate, the synthesis of phosphocreatine in preparations from alloxanized rats is significantly less than in those from normal animals. Insulin injected into the alloxanized rats prior to tissue sampling was found to promote an increased formation of phosphocreatine. Similar results have been obtained when malate and pyruvate are substituted for succinate in the homogenates. Insulin had no appreciable effect on the oxygen consumption in these experiments. The results indicate an action of insulin in increasing the efficiency of oxidative phosphorylations and the possible participation of insulin in reactions involving the tricarboxylic acid cycle.

Relation of the adrenal cortex to the increased macrophagic activity induced by starvation. ALBERT S. GORDON and GRACE F. KATSH (by invitation). *Dept. of Biology, Washington Square*

College of Arts and Science, New York Univ., New York City. It has been reported (Gordon and Katsh, *Federation Proc.* 7: 42, 1948) that chronic inanition results in an increased phagocytic response of the macrophagic system towards colloidal substances, particularly thorium dioxide. Experiments were designed to test the possibility that this starvation effect is mediated through the adrenal cortex. Thirty male rats (150–200 gm.) were subjected to chronic inanition (5 gm. ration daily) for 25–30 days. A body weight loss averaging 31% was experienced at the end of this time. Of these 30 animals, 18 were adrenalectomized and 12 served as controls. Each of 8 of the adrenalectomized animals was injected with a total of 20 cc. of aqueous adrenal cortical extract (Upjohn) and 1 cc. of lipoadrenal extract (Upjohn), distributed over a 3-day period before, and 30 hr. after adrenal removal. The other 10 adrenalectomized rats were injected with 21 cc. of 1% saline, spaced in the same manner as above. The 18 adrenalectomized animals (used 1 day after the operation), the 12 unoperated starved and 10 normal fed rats were injected intravenously with 0.3 cc. thorotrast and killed approximately 20 hr. later. Mean values ± standard errors for ThO_2 per gm. dried spleen were: *1*) fed untreated, 58.2 ± 7.8 mg.; *2*) starved untreated, 175.5 ± 11.3 mg.; *3*) starved adrenalectomized given saline, 70.6 ± 7.1 mg.; *4*) starved adrenalectomized given adrenal cortical extract, 123.5 ± 13.4 mg.

Histological studies of the spleens revealed that the increased numbers and marked activity of the macrophages, characteristic of the starved rat, were diminished by adrenalectomy and that adrenal cortical hormone, but not saline, was greatly effective in preventing this impaired condition in the adrenalectomized animal.

Effect of insulin on the rate of oxygen consumption by frog muscle. Desmond R. H. Gourley (by invitation) and Kenneth C. Fisher. *University of Toronto, Toronto, Canada.* In the presence of citrate, succinate and acetate, insulin causes an increase in the rate of oxygen consumption by isolated intact frog muscle. The effect of the citrate, succinate, and acetate appears to be correlated with the ability of these compounds to produce spontaneous contractions in the muscle.

Experimental observations on neural mechanisms involved in itching. David T. Graham and Helen Goodell (introduced by Harold G. Wolff). *New York Hospital and the Depts. of Neurology and Psychiatry, Cornell Univ. Medical College, New York City.* Itching was produced by application of cowhage to skin. Two components could be distinguished in the resulting itch: a superficial pricking and a deeper burning sensation. Ischemia of the forearm, causing loss of pricking pain but not of deep burning pain, abolished the superficial pricking component, but not the deeper burning component. Procaine block of a cutaneous nerve, resulting in the abolition of burning pain from pin prick with retention of pricking pain in an area of incomplete anesthesia, prevented development of burning itch, but allowed pricking itch to develop. It is suggested that the two qualities of itch are mediated respectively by the rapidly and slowly conducting pain fibers from the skin. Pin pricks in an itching area, or in areas adjacent to it, or in the same dermatome as the itching area, even at a distance of 30 cm., abolished itching for 20 to 60 seconds. In a zone of secondary hyperalgesia (developing after faradic stimulation over a cutaneous nerve), cowhage induced either pain or no sensation, but not itch. The evidence cited above indicates that to understand the neural mechanisms of itching it is necessary to consider not only peripheral phenomena, but also processes in the central nervous system, presumably in the cord.

Early stages of gastric surface epithelization. Rhoda Grant (introduced by A. C. Ivy). *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Gastric surface epithelization uncomplicated by concurrent regeneration of glandular epithelium has been studied histologically (1–8) hr. after *a*) mild irritants have removed the surface cells; *b*) minute superficial cuts. Irritants were used *1*) in cats with gastric fistula under acute conditions, or were fed *2*) to cats by tube; and *3*) to rats by tube. Control cuts were biopsied immediately after infliction and compared with cuts allowed to heal for varying intervals up to 8 hr. Serial sections were stained with hematoxylin and eosin or Mayer's mucicarmine.

In the early stages of surface regeneration, the cells which restore the surface continuity in denuded areas include parietal cells. These are most numerous in the walls of crypts reforming at the surface level, but occasionally may be seen on the free surfaces between regenerating crypts and in new surfaces forming in cuts at or below midgland level. The majority of cells in the new surfaces stain faintly for mucus and may be from the bases of crypts not completely removed by the irritant, and/or mucous neck cells. Evidence points, as in former experiments, to restoration of surface continuity and of the essential surface topography before proliferation replaces cells shifted to the surface.

Effect of ethyl carbamate on temperature regulation. Ronald Grant and Marilyn E. Robbins (introduced by Victor E. Hall). *Dept. of Physiology, Stanford Univ., Stanford University, Calif.* Magoun (*Proc. Soc. Exp. Biol. Med.* 37: 711, 1938) showed that polypneic panting occurs at normal

or subnormal body temperatures in some cats anesthetized with urethane. We have investigated the possibility that urethane promotes heat loss by an action on the thermoregulatory centers. Rabbits anesthetized with urethane (1.0 to 1.5 gm/kg. in 20% solution intraperitoneally) show a rapid drop of body temperature due to: *1*) decreased heat production; *2*) vasodilation in the ears; *3*) in a few cases, polypnea. After body temperature has fallen 1 to 3°C. polypnea ceases, vasoconstriction occurs and the fall of temperature is retarded. Shivering may occur. Warming restores the vasodilation and incites polypnea usually at subnormal rectal temperatures. Despite this evidence that urethane lowers the temperature thresholds for activation of thermoregulatory mechanisms we have concluded that its action is probably not upon the primary centers of thermoregulation for the following reasons: *1*) whereas vasodilation and polypnea are practically coincident in normal animals urethane usually slows respiration (rather than causing polypnea), while inciting vasodilation, and thresholds for activation are widely separate in warmed animals; *2*) intravenous or subcutaneous injection rarely causes vasodilation or polypnea; *3*) inhibition of heat loss mechanisms by bacterial pyrogens is unaffected by previous or subsequent intravenous injection of urethane; intraperitoneal injection may reverse the inhibition of vasodilation without affecting inhibition of polypnea.

Influence of repeated artificial pneumothorax on hematocrit values and plasma volume. WILSON C. GRANT (introduced by WALTER S. ROOT). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* Pneumothorax was maintained for 20 to 80 days in 10 unanesthetized dogs, 3 of which were splenectomized. One hundred to 500 cc. of air were injected at intervals of from 2 to 5 days. The volume of the refill was always adjusted to maintain the desired elevated intrathoracic pressure (−2 mm. Hg to +2 mm. Hg). Pneumothorax was moderate and the animals remained in good condition. Measurements were made on jugular venous blood during a 2- to 3-weeks control period and at 2- to 3-day intervals in the pneumothorax period, at least *2 days following an insufflation*. Hematocrit and O_2 capacity values increased 10 to 30% in 6 to 20 days. Plasma volume (T-1824) decreased 6 to 28% while the refractive index of plasma rose slightly. No significant increase in reticulocyte percentage was observed. Approximately the same response was observed in the splenectomized dogs. Blood values returned to those of control level within 1 to 2 weeks after cessation of pneumothorax. Anoxic anoxia was not an important factor, since normal values for arterial O_2 saturation were obtained in 18 out of 20 measurements on 5 dogs during the pneumothorax period. The animals responded to partial collapse by an increased pulmonary ventilation. The response of the blood to repeated pneumothorax is, therefore, a relative polycythemia which can be partially explained by plasma loss. No clear evidence of increased erythropoiesis was found. Hemoglobin production following pneumothorax is being studied by the Whipple-Robbscheitt-Robbins technique on the chronically anemic dog.

Non-dependence of tubular sodium reabsorption upon glomerular function. D. M. GREEN and ALFRED FARAH (by invitation). *Depts. of Medicine and Pharmacology, Univ. of Washington, Seattle, Wash.* The amount of sodium reabsorbed by the tubular apparatus has been considered to represent a constant fraction of the amount filtered (Mokotoff *et al.* (*Journal Clin. Invest.* 27: 1, 1948). Such a hypothesis would make the excretion of sodium primarily dependent upon glomerular function. However, the ability of many patients to maintain sodium balance despite markedly impaired filtration rates would appear more consistent with the supposition that the percentage of filtered sodium rejected by the tubular apparatus could be regulated independently of the filtration function. Evidence for the existence of such an independent regulation has been secured by measuring the percentage of filtered sodium excreted under different conditions of sodium load. Solutions of sodium chloride varying in concentration from 0.86 to 30% were infused into dogs at rates of 1 to 9 cc/min. Under such circumstances the test animals were found capable of increasing sodium excretion several 100-fold, to values which represented as much as 40% of the quantity of sodium filtered. These changes were not accompanied by consistent or proportionate alterations in glomerular filtration rate or renal plasma flow. Calculations based on the clearance data indicated that the rise in sodium excretion was due almost entirely to a decrease in the percentage of its tubular reabsorption.

Effects of adrenal resection on hypertension and diabetes. D. M. GREEN, J. N. NELSON (by invitation) and G. A. DODDS. *Dept. of Medicine, Univ. of Washington School of Medicine, Seattle, Wash.* Bilateral adrenal resection was performed on a 29-year-old female suffering from hypertension and diabetes. Diabetes had been present since age 6. Daily insulin requirements were 70 to 85 u. Hospitalization for diabetic complications had occupied almost one quarter of the last 3 years. Hypertension, first recognized 8 years previously, had become severe and progressive. Transitory episodes of encephalopathy and myocardial failure characterized the last year. Preoperative blood

pressure averaged 260 mm. Hg systolic, 140 mm. diastolic. The heart was enlarged on X-ray. Renal function was approximately 40% of normal. Grade-4 retinitis was present. At operation, performed in 2 stages, not less than 98% of all adrenal tissue was removed. The immediate postoperative period was marked by paralytic ileus and transitory myocardial failure. Later complications included marked weakness, intermittent vomiting and one episode of frank crisis. The patient became ambulant during the 2nd week and was discharged on the 22nd postoperative day. Blood pressure fell to 110 mm. Hg systolic, 80 mm. diastolic 4 hr. postoperatively. Elevated pressures were subsequently observed only during brief periods of desoxycorticosterone overdosage. X-ray 3 weeks postoperatively showed normal heart size. Cortical extract requirements initially exceeded 200 cc/day but decreased progressively during convalescence. Insulin needs roughly paralleled cortical extract dosage. Six weeks postoperatively the blood pressure was normal, the daily extract requirement was 15 cc. and the insulin requirement 25 U.

Variation of choline acetylase content of brain in stressed and unstressed normal and adrenalectomized rats. RUVEN GREENBERG (introduced by F. A. HARTMAN). *Dept. of Physiology, Ohio State Univ., Columbus, Ohio.* In adrenal cortical insufficiency there is a generalized weakness whose mechanism is unclear. The nerve tissue conduction and maintenance is in some way involved. The choline acetylase content of acetone-dried brain powders made of pooled rat brains was measured by the rate at which the powders acetylated choline to form acetyl choline (method of Nachmansohn and Machado, 1943). The acetylations by brain extracts of adrenalectomized animals, stressed and unstressed, compared to sham-operated animals treated in the same way gave variable values in all the groups tested. The range of values between the adrenalectomized and sham groups and the stressed and unstressed groups overlapped. The effect of coenzyme (Lipmann, 1945) added to the acetylation substrate made for about 100% increase in synthesis. This increase did not vary significantly between the different preparations. The effect of adrenalectomy and stress, individually or combined, on brain tissue does not consistently change either the choline acetylase or coenzyme levels. These act on another part of the acetylcholine mechanism or on other mechanisms entirely.

Comparison of effects of a new ganglionic blocking agent and a new sympatholytic. K. S. GRIMSON and J. R. CHITTUM (by invitation) and F. H. LONGINO. *Dept. of Surgery, Duke University, Durham, N. C.* Similar studies have been performed testing 2,6 dimethyl diethyl piperidinium Bromide (S.C. *1950*) and 2-(N,p-tolyl-N-(m'oxyphenyl)-aminomethyl)-imidazoline hydrochloride (C. *7337*) in dogs and patients. In dogs effective doses of *1950* ranged from 5 to 10 mg/kg. and of *7337* from 0.2 to 0.5 mg/kg. These amounts reduced blood pressures, prevented increase of pressure with occlusion of carotid arteries, prevented increase of blood pressure with anoxia, and reduced or blocked increase of blood pressure normally following stimulation of the central end of a divided vagus nerve. Pituitrin increased blood pressure after effective doses of either drug. Differences between the two drugs were apparent in other tests. Pulse rate decreased or did not change with *1950* and increased temporarily with *7337*. Increase of blood pressure following adrenalin was accentuated after *1950* and blocked after *7337*. With *1950* an increase of blood pressure occurred one or two minutes after discontinuing anoxia. This did not occur after *7337*. Prostigmin following *1950* elevated blood pressure and restored responsiveness to vasomotor reflexes, but not following *7337*. Chronic neurogenic hypertension in dogs was reduced by either drug. Increase of blood pressure with increased intracranial pressure was not prevented by effective doses of *1950*, but was reduced or prevented by large doses of *7337*. One to two mg/kg. of either *1950* or *7337* injected intravenously in patients or 1 to 2 mg/kg. of *7337* orally usually blocked increases of blood pressure with breath holding or with one hand in ice water, and warmed extremities. Results of additional clinical studies demonstrating differences of effects in patients will be presented.

Effect of spinal cord transection on blood acid-base balance during exercise. FRED S. GRODINS and DONALD P. MORGAN (by invitation). *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, Ill.* Blood acid-base balance was studied in barbitalized dogs during electrically induced exercise of the hind limbs before and after section of the spinal cord at T10. The total CO_2 content and *p*H of arterial plasma were determined at rest and during a steady state of exercise, and the corresponding CO_2 tensions calculated from the Henderson-Hasselbalch equation. Percutaneous electrical stimulation with 60-cycle AC modulated at 100/min. was used to produce oxygen consumptions of 2–7 times the resting value. In 11 experiments on 8 dogs, there was an average fall of 1.77 mm. Hg in arterial pCO_2 during a steady state of exercise compared to the resting value. This mean difference was not significantly different from zero ($t = 1.65$; $P > 10\%$). In 14 experiments in 6 dogs after cord section, there was a mean increase of 3.94 mm. Hg in arterial pCO_2 during a steady state of exercise which was highly significant ($t = 4.59$; $P < 1\%$). Oxygen consumptions during exercise

varied from 2.2 to 5.5 times the resting level in both groups. Plots of the acid-base pathways revealed essentially no change in the intact group for oxygen consumptions up to 2.5 times resting. Above this level, a metabolic acidosis pathway was followed. The sectioned group followed a pathway corresponding to a respiratory acidosis with a metabolic acidosis superimposed.

Distribution of microgram amounts of labeled thyroxine in the rat. J. GROSS and C. P. LEBLOND (introduced by S. M. FRIEDMAN). *Dept. of Anatomy, McGill Univ., Montreal, Canada.* Microgram amounts of labeled thyroxine were prepared by iodinating DL-diiodothyronine on a micro-sale or by extracting thyroxine from the plasma of rats previously given radio-iodine. The purity of the material was checked by isotope dilution. The distribution studies at 2, 24 and 72 hours after injection emphasized the predominant role of the liver in storing thyroxine and excreting it into the bile. Thyroxine is then mostly eliminated in the feces. In addition, large amounts are present in muscle and skin. These conclusions probably apply to the normal thyroid hormone because it was possible to demonstrate the presence of a thyroxine-like compound in thyroid tissue, plasma, liver and muscle under normal conditions.

Mucoproteins of gastric juice and mucus and the mechanism of their secretion. ALLAN GROSSBERG (by invitation), S. A. KOMAROV and HARRY SHAY. *Samuel S. Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Pa.* Evidence has been obtained in support of earlier observations (Webster and Komarov, *J. Biol. Chem.* 96: 133, 1932) that at least two different mucoproteins are secreted by the gastric mucosa in the dog. One of the mucoproteins is characterized by the presence of both hexuronic acid and hexosamine, apparently in the proportions found in mucoitin sulfuric acid, and it is present both in alkaline mucus and in acid gastric juice; the second mucoprotein has hexosamine in its molecule but a much smaller proportion of hexuronic acid, if any at all. The prosthetic group of this latter mucoprotein may be identical with the 'neutral polysaccharide' described by Meyer, Smyth and Palmer (*J. Biol. Chem.* 119: 73, 1937). This mucoprotein is particularly characteristic of the alkaline mucus although it is also present in solution in acid gastric juice. Data were obtained on gastric secretions from dogs *1*) in the resting state; *2*) prior and subsequent to sham-feeding and *3*) prior and subsequent to the injection of histamine. The analytical results indicate that *1*) the secretion of the two mucoproteins is stimulated by sham-feeding but to different degrees, suggesting some difference in the secretory mechanisms involved; *2*) the secretion of the two mucoproteins does not parallel that of either pepsin or acid; *3*) the effect on secretion with increased nervous stimulation is greatest for pepsin and the least for the hexuronic acid-free mucoprotein, with an intermediate effect for the other mucoprotein; *4*) histamine does not stimulate the secretion of either mucoprotein.

Experimental production of hemorrhages in dogs with the use of aminopterin and their control with toluidine blue. BURTON J. GROSSMAN, MARGARET H. SANDERSON, and WILLADENE EGNER (introduced by J. Garrott Allen). *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* The incidence of hemorrhage in patients with acute leukemia appears to be increased during the early period of aminopterin (4-aminopteroyl glutamic acid) therapy. The bleeding tendency is not present when the patient is in complete remission. Studies of the effect of aminopterin on the clotting mechanism in the normal dog were undertaken. These included the whole blood clotting time, prothrombin time, platelet count, observation for lysis of clot, the amount of protamine required to allow standard heparinized samples to clot and gross observations for the absence or presence of bleeding. Ten dogs, weighing 8 to 10 kg., were given a daily dose of 0.5 mg. of aminopterin. Bleeding from the mouth and gastrointestinal tract occurred in all animals between the 8th and 11th day. At this time the platelet count was rarely below 150,000. The clotting times were normal or slightly prolonged. The prothrombin activity was unchanged except just before death. Increased lysis was not observed. The protamine requirement of standard heparinized blood was increased and this increase was out of proportion to the slight increase in the whole blood clotting time. Aminopterin was discontinued in 2 dogs and bleeding stopped after 4 days. Two dogs were given a single intravenous dose of toluidine blue (5.0 mg/kg. of body weight) and bleeding stopped within 6 hr. Six dogs were continued on aminopterin and bleeding was controlled with a single injection of toluidine blue. The protamine requirement returned to normal. These animals died within 3 days without external evidence of hemorrhage, although 2 showed a hemorrhagic gastritis.

Clinical and physiological studies on thiomerin—a subcutaneously injectable mercurial diuretic. J. GROSSMAN (by invitation), R. E. WESTON, I. S. EDELMAN (by invitation) and L. LEITER (by invitation). *Medical Division, Montefiore Hospital, New York City.* Approximately 200 subcutaneous injections of Thiomerin (Di-sodium-N(γ-carboxymethylmercaptomercuri-β-methoxy) propyl camphoramate) in more than 40 patients produced changes in body weight and daily urinary volume and chloride excretion comparable to those following the injections of Mercuzanthin and Mercu-

hydrin. No systemic reaction or unusual local discomfort was observed. Only two reactions occurred at the site of injection; one consisted of ecchymosis in a patient with a bleeding diathesis and the other, of necrosis and sloughing of skin when Thiomerin was administered into an area of dependent edema. Both patients tolerated subsequent injections without ill-effect. In addition, simultaneous renal clearances of mannitol, PAH, sodium, chloride and uric acid, prior to and following intravenous Thiomerin or Mercuzanthin administration were determined. Except for the transient renal hemodynamic response to the Theophylline fraction of the Mercuzanthin, no significant differences between the diuretic effects of the two drugs were noted. In collateral studies on other tubular functions, both mercurials were found to have qualitatively and quantitatively similar depressive actions (i.e. decreased glucose T_M and renal PAH extraction). A comparison between the effects of intravenous and subcutaneous administration of Thiomerin in the same patients revealed no significant differences except in the time of response.

Action of neurohumoral agents in the human skin. M. I. GROSSMAN, HENRY D. JANOWITZ (by invitation) and R. R. SONNENSCHEIN (by invitation). *Dept. of Clinical Science, Univ. of Ill. College of Medicine, Chicago, Ill.* Physiological activities known to be mediated by neurohumoral agents are sweating by acetylcholine, piloerection by sympathin, and constriction of small blood vessels by sympathin. Intradermal injection of neurohumoral agents, in addition to stimulation of the effector organs just listed, stimulates certain nerve endings. These include *a*) sensory nerve endings for pain and itch which respond to histamine, *b*) motor nerves to the sweat glands and piloerector muscles which respond to acetylcholine and to nicotine, and *c*) certain dorsal root ramifying systems of nerves involved in vasodilation, hyperalgesia and itchy skin, all subject to stimulation by histamine. The effects under *b*) and *c*) can be classified as axon reflexes and the drugs listed with them are capable of initiating the reflex. The neurohumoral effector agent of these axon reflexes is known in the case of *b*) but not of *c*). The nicotinic effects can be blocked by tetraethylammonium (TEA). TEA also blocks the initiation of the axon reflex itchy skin surrounding a histamine wheal but it does not interfere with the local sensation of spontaneous itch. Pontocaine causes local anesthesia concomitantly with surrounding flare and hyperalgesia, suggesting that it selectively anesthetizes pain nerve endings but stimulates, directly or indirectly, nerves concerned in establishment of hyperalgesia and flare. The results with TEA in itchy skin and pontocaine in hyperalgesia each demonstrates dissociation between the local primary sensation and the altered state of sensibility surrounding the injection site, thus supporting the hypothesis that the nerve fibers responsible for the altered state are not the same ones which convey the impulses for the spontaneous sensation.

Pressure changes in the pulmonary artery and aorta before and after ligation of the patent ductus arteriosus. ROBERT F. GROVER (by invitation), HENRY SWAN II (by invitation) and CLARENCE A. MAASKE. *Depts. of Physiology and Pharmacology, and of Surgery, Univ. of Colorado Medical Center, Denver, Colo.* Measurements of pulmonary artery and aortic blood pressures by puncture of the vessel at operation were made by means of resistance type strain gauges in 7 patients just before and after ligation of patent ductus arteriosus. Cardiac catheterization was employed in some of these patients and comparisons between pulmonary arterial blood pressure before and after opening the thorax were made. Immediately following ligation of the patent ductus arteriosus, the mean pulmonary artery pressure fell an average of 11 mm. Hg for all patients. Excepting one patient, pulmonary artery pulse pressure was unchanged or only slightly increased. Following ligation of the duct, mean aortic pressure rose an average of 9.5 mm. Hg, with an associated decrease in pulse pressure and an elevation of diastolic pressure. The contours of the pulse waves obtained while the ductus was patent were distorted in both the pulmonary artery and the aorta, probably resulting from pressure irregularities accompanying the abnormal blood flow. Ligation of the ductus generally gave pulse waves which approached the normal by reduction of these distortions. Good correlation was seen between the pulmonary artery pressures obtained by catheterization with the chest closed and those obtained by needle puncture with the chest open. More extensive comparisons of the normal pressures under these two conditions have been conducted on dogs.

Excitability changes in dorsal roots produced by electrotonic effects from adjacent afferent activity. HARRY GRUNDFEST and JONATHAN MAGNES (by invitation). *Dept. of Neurology, College of Physicians & Surgeons, Columbia Univ., New York City.* Electrical activity produced in the spinal cord of cats by stimulating a dorsal root induces electrotonic potentials in an adjacent, inactive root. According to Lloyd and McIntyre (*Federation Proc.* 7: 74, 1948), the first three phases, beginning negatively, are referable to the intramedullary afferent volley. Following, and overlapped by the terminating negative cord potential, they found a positive phase which is "an electrotonic extension of the potential of terminating collaterals" of the active root, with 3 msec.

"half-life." This sequence of electrotonic potentials in the inactive root should cause modifications of excitability. Stimulating electrodes on a root, close to the cord, tested the temporal changes of excitability during cord activity produced by conditioning stimulation of another root (cats, nembutal anaesthesia). Excitability was heightened during the first half msec. after the conditioning volley. Depression followed, lasting about 3 msec. (maximum between 1.5 and 2.5 msec.). Though of shorter duration, this depression is consonant with the existence of the positive electrotonic phase of Lloyd and McIntyre, and further demonstrates its reality. The briefer duration may be ascribed to the rapid decay of the magnitude of the electrotonic potential in the extramedullary root. These excitability changes have been observed in approximately 50% of the root pairs tested. Variability is to be expected since the effect depends upon appropriate spatial relations between the intramedullary terminations of active and inactive fibers.

Action of antitrypsin and antifibrinolysin on the first phase of the blood clotting reaction. M. MASON GUEST and THOMAS E. NELSON (by invitation) *Dept. of Physiology and Pharmacology, Wayne Univ. College of Medicine, Detroit, Mich.* The proteolytic enzyme, trypsin, is inactivated by either soy bean trypsin inhibitor or beef plasma antifibrinolysin (antiplasmin). Similarly, fibrinolysin (plasmin) is inactivated by both trypsin inhibitor and antifibrinolysin. However, it has been found that only one of these inhibitors modifies the first phase of the blood clotting reaction. Soy bean antitrypsin (crystalline material prepared by M. Kunitz or the E. C. Loomis preparation) inhibits the first stage of the clotting reaction but antifibrinolysin (E. C. Loomis preparation) has no demonstrable effect upon the rate or character of clot formation. The failure of antifibrinolysin to alter the clotting reaction suggests that this substance is different from soy bean antitrypsin and that proteolytic activity involving fibrinolysin is not a part of the clotting mechanism. Investigation of the mode of action of antitrypsin in inhibiting the first phase of the clotting reaction has failed to reveal whether this substance acts as an antithromboplastin, an antiprothrombin, an anti-accelerator globulin or whether it acts by blocking the reaction of these substances. Unless an inhibitor of thrombin formation can be shown to react at a measurable rate with one of the essential components of the first phase of the clotting reaction, reducing the potency of this component as a participant in the thrombin formation reaction, it does not appear possible to determine the point of action of such an interfering substance with methods available at present.

A chromogenic steroid occurring in the urine of males after muscular exertion. H. S. GUTERMAN and L. M. KRAUS (introduced by R. Levine). *Dept. of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* In performing pregnandiol determinations on urine samples from normal adult males according to the method previously published from this laboratory (*J. Lab. & Clin. Med.* 33: 356, 1948), we found unusually large amounts of 'pregnandiol' present in some of the specimens. Study of the chromogen revealed that on extraction it is present in the neutral non-ketonic steroid fraction when separated with Girard's reagent T. Precipitation with digitonin indicated that a β-hydroxyl group might be present in the *3* position. Since the Zimmermann reaction produced no color with the material after digitonin precipitation, it is believed that the presence of ketonic groups in *position 3, 17* and *20* can be ruled out. Bromine water was decolorized by an alcoholic solution of the material, and thus the presence of at least one double bond was indicated. Thinking that the product might be 5:6 androstenediol (3β-17α), we applied Bostock's fluorescent test (*Nature* 162: 577, 1948). The material under study gave a green-blue fluorescence instead of the violet shade emitted by crystalline androstenediol. Further studies are being conducted to characterize this urinary excretion product. To date, the chromogen has been found only in males. It appears to be of significance physiologically since it occurs in the urine in rather large amounts following muscular exertion.

Lipide studies on whole anterior lobe, nuclei, and cytoplasmic particulates of lamb pituitary glands. M. H. HACK (introduced by HUBERT R. CATCHPOLE). *Dept. of Pathology, Univ. of Illinois, College of Medicine, Chicago, Ill.* Lyophilized whole anterior pituitary of lamb and isolated cell fractions were assayed for free and protein-bound phosphatides according to the procedure described earlier (*J. Biol. Chem.* 169: 137, 1947). All cell fractions were obtained from homogenates prepared by Claude's method. The large granule fraction was clearly separable into a buff-colored upper layer (mitochondria) and a white lower layer (eosinophilic granules). The identity of all fractions was confirmed histologically. A new "acetal" was shown, by means of fuchsin sulfurous acid, to be present in the lipides extracted from the intact tissue and from each of the cell fractions by acetone, chloroform-methanol (1:1), and by benzene. Histochemical confirmation was effected by means of the same technique applied to frozen sections of fresh tissue. Acetal phosphatide was barely detectable. The distribution of lecithin, cephalin, and sphingomyelin, expressed in μM/gm. dry weight, and the relative concentration of "acetal" were:

	LECITHIN	CEPHALIN	SPINGOMYELIN	'ACETAL'
Whole tissue	75	58	17	+
Mitochondria	117	87	46	++
Eosinophilic granules	62	54	29	+
Microsomes	162	156	40	++++
Nuclei	43	38	0	+

One-third to one-half of both lecithin and cephalin were present as lipoprotein. The sphingomyelin was largely free except in the microsome fraction. As in the case of cell fractions of rat liver, each fraction is here shown to be distinct in its phosphatide composition.

Myocardial metabolism in thiamine-defficient dogs studied by intravenous catheterization of the coronary sinus. D. B. HACKEL, W. T. GOODALE, R. P. JOHNSON (introduced by W. FLEISCHMANN). *Physiology Section, Medical Division, Army Chemical Center, Md.* Studies of the myocardial utilization of lactate and pyruvate were made in intact normal dogs and in dogs with acute and chronic thiamine deficiency, using the technique developed by Goodale et al. (*Am. J. Physiol.* 152: 340, 1948) for catheterizing the coronary sinus. Mean arterial pyruvate levels of 1.71 ± 0.13 mg. % with coronary A–V differences of 0.76 ± 0.1 mg. %, and lactate levels of 12.2 ± 0.96 mg. % with coronary A–V differences of 5.3 ± 0.5 mg. % were obtained in a series of normal nembutalized dogs.

Thiamine deficiency was produced by feeding a diet of canned dog food which had been autoclaved at 15 lb. pressure for 12 hours to destroy thiamine, and supplemented by other synthetic vitamins of the B complex as well as Vitamin C. Anorexia and weight loss began in about 3 weeks, going on to convulsions with opisthotonus in 6 weeks. Complete relief of symptoms was brought about by the intravenous administration of 2 mg. of crystalline thiamine. Chronic thiamine deficiency was produced by supplementing the above diet with daily intramuscular injection of 50–100 γ of crystalline thiamine. Small additions to, or subtractions from, the daily dose of thiamine were reflected in prompt weight changes. Acute deficiency resulted in decreased pyruvate utilization by the heart, with arterial levels up to 5.9 mg. %, and almost no lactate utilization, despite arterial levels up to 60 mg. %. Determinations done in the course of chronic deficiency revealed an uptake by the heart of both lactate and pyruvate which was below the expected normal range.

Effects of shock (low arterial blood pressure) on the pulmonary vessel pressures. FRANCIS J. HADDY (by invitation), GILBERT S. CAMPBELL (by invitation) and M. B. VISSCHER. *Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* Studies have been made on 64 dogs anesthetized with nembutal, 30 mg/kg., on pulmonary artery and venous pressures measured with a Statham strain-gauge attached to flexible catheters. The average mean pulmonary artery and venous blood pressure measured relative to the intrathoracic pressure in 57 animals with peripheral arterial blood pressures above 100 mm. Hg was 17.8 and 8.5 mm. Hg respectively. In 7 animals with blood pressures below 100 mm. Hg the pressures were 14.0 and 2.3 mm. Hg respectively. Separating the group of 57 animals into those with peripheral arterial blood pressures between 100 and 150 mm. Hg and those with pressures above 150 mm. Hg one finds the pulmonary arterial and venous pressures in the former 40 animals to be 16.7 and 7.6 mm. Hg respectively and in the latter 17 animals to be 18.5 and 9.7 mm. Hg respectively. From these data there appears to be a positive correlation between the peripheral arterial blood pressure and the pulmonary pressures. From the same group of animals there was found to be no significant difference in the pulmonary pressures in males or females. When broken down into animals with body weights below 20 kg. and those above 20 kg., there was noted no significant difference in pulmonary arterial or venous pressures in relation to weight. It is likely that a decreased cardiac output in shock is at least partly responsible for the observed effects.

Light flux constancy at the intensity limin of the eye for total receptor cross-sectional areas. CHARLES HAIG. *Dept. of Physiology and Biochemistry, New York Medical College, New York, N. Y.* By methods previously described (*Federation Proc.* 7: 47, 1948) it has been demonstrated that for homogeneous rod populations, i.e., for specific retinal regions, the product of the threshold intensity I and the retinal (stimulus) area A is constant for areas ranging from 0.04 degree subtense to 1.0 degree subtense. For homogeneous cone populations IA is constant for areas up to 0.5 degree subtense. Calculations have been made for a single eye to determine whether the luminous flux is constant for rod and cone cross-sectional areas in non-homogeneous retinal regions, e.g., the fovea as compared with peripheral regions. The data of Østerberg (1935) and Polyak (1941) on rod and cone diameters and population densities in various retinal regions provide the means for computing A in terms of total rod or cone cross-sectional area in a given retinal (stimulus) area. For rods it is found that IA so computed is constant from 2 degrees out to 6 degrees from the foveal center. From 10 out to 18 degrees IA is again constant at twice the value found closer to the center. For the inner cone segments it is found that IA is constant from the foveal center out to 10 degrees in the periphery, beyond which it rises to a value twice as great at 18 degrees in the periphery. These calculations for

one eye indicate that measurements on several eyes will reveal that the luminous flux actually incident upon the receptor elements is constant for the entire central area.

Oxygen consumption of liver slices from febrile and normal rabbits. V. E. HALL, J. FISHGOLD (by invitation) and R. GRANT (by invitation). *Dept. of Physiology, Stanford Univ., Stanford, Calif.* Observations published in the literature suggest that during the early stage of fever the temperature in or near the liver rises earlier and in greater degree than the rectal temperature. This has been interpreted as showing that an increase in liver metabolism contributes significantly to the febrile temperature rise. However, measurement *in vitro* of the O_2 consumption of liver slices taken from rabbits 15 minutes after the injection of typhoid-paratyphoid vaccine (a time when the liver temperature was said to have been rising) yielded a mean Q_{O_2} value of 3.71 as compared with a mean of 4.32 for untreated controls. The difference is statistically significant and shows that the liver metabolism under the conditions employed is depressed in tissue taken from febrile animals. If the heat production of the liver is increased early in the fever produced by bacterial pyrogen, it is attributable to factors inoperative when the liver metabolism is studied *in vitro*.

Studies of a case of congenital heart disease with cyanotic episodes. W. F. HAMILTON, J. A. WINSLOW (by invitation) and W. F. HAMILTON, JR. (by invitation). *Depts. of Physiology, Medicine and Roentgenology, Univ. of Georgia School of Medicine, Augusta, Ga.* The patient, an 18-year-old girl had been through grammar school and was in fair health though she had been subject to spells of cyanosis and fainting. Clinical impression was Tetralogy of Fallot. During two cardiac catheterizations she became intensely cyanotic and irrational but not unconscious. Pressures in vein and auricle were normal. Rt. ventricle 100/0, Aorta 65/30, Femoral 85/40, Pul. art. 5/0. These data confirm patency of the interventricular septum and pulmonary stenosis. Blood in right ventricle, pulmonary artery and femoral artery were 10–20% saturated with A–V difference between 0 and 1%. Oxygen consumption varied around 200 cc/min. though for 2 minutes it apparently ceased. When not cyanotic O_2 consumption was steady at 150 cc/min with blood pressure 140/80 and arterial O_2 saturation of 70–80%. Cardiac index at this time was 2.04 L. and circulation time 14 sec. by dye injection method. Cyanotic episodes resulted from activity, emotion, or vasodilator drugs and were accompanied by lowered arterial pressure, oxygen saturation, and O_2 uptake.

Various explanations may be considered. The favored one is that the functionally single ventricle pushes blood out into the two circulations as governed by their respective resistances. In this case the pulmonary resistance seems to be very high and a lowering of the systemic resistance causes increasing amounts of the ventricular output to pass out of the aorta and less or even no blood to perfuse the lungs. This caused temporary reduction or even cessation of oxygen uptake and varying degrees of arterial unsaturation.

Measurement of cardiac volume in the dog from X-ray shadow area. W. F. HAMILTON, JR. (by invitation) and PHILIP DOW. *Depts. of Physiology and Roentgenology, Univ. of Georgia School of Medicine, Augusta, Ga.* An effort has been made to standardize a cardiac-volume method for use during experiments on circulatory dynamics. After sacrifice experiments, P-A roentgenograms were taken of the closed chests of 53 supine dogs. The chests were opened immediately, the great vessels were tied off, and actual cardiac volumes were measured by immersion. These volumes were compared with the areas of corresponding shadows on the films (corrected for tube distance).

No formula found for dog or human hearts approximated the relationships obtained, so a new formula was empirically derived. The best fit was given by an equation of the form: Vol. = $a\left[\left(\frac{s}{l}\right)^{\frac{1}{2}} \times \text{area}\right]^{b}$, in which s is the shorter and l the longer dimension of the shadow. Parallel projection of a prolate spheroid should give $a = 0.75$ and $b = 1.5$. The oversimplifications in this hypothesis are indicated by the empirical results: $a = 0.4$ and $b = 1.7$. This formula predicts the volume of dog hearts larger than 100 cc. with an average error of 5% (maximum 13%). Predictions for smaller hearts are consistently too high or too low (average error 22%). Most of these, however, were from puppies and do not fall in the range of animals ordinarily used in the experiments. Supplementary projections were not employed to increase accuracy, since such would not be feasible during experiments for which the method is devised.

Effects of di-isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity. J. L. HAMPSON (by invitation), C. F. ESSIG (by invitation), ALICE WILLIS (by invitation), and H. E. HIMWICH. *Medical Division, Army Chemical Center, Md.* Di-isopropyl fluorophosphate (DFP) was injected into one common carotid artery of curarized rabbits receiving artificial respiration. A needle electrode was inserted through the skull over each cerebral hemisphere. The electroencephalographic changes were observed with the Grass apparatus and cholinesterase activities of various parts of the brain were subsequently determined by the method of Michel.

The cerebral parts included the cortex, midbrain, medulla and cerebellum. The electroencephalographic changes were placed in five categories of increasing severity: *1*) No permanent change from the control. *2*) Early permanent, alterations including slight increases in regularity, amplitude and rate. *3*) Intermediate, phase 1, marked changes limited however chiefly to the right hemisphere. *4*) Intermediate, phase 2, marked changes (short of grand mal) appearing in both cerebral hemispheres. *5*) Continued grand mal-like activity on both sides.

In general, with succeeding categories, cholinesterase activity decreased more profoundly until the fourth category was obtained. Statistical analyses of cholinesterase activities revealed however no differences between no permanent and early permanent. But between early permanent and intermediate phase 1 all differences were significant while between intermediate phase 2 and grand mal no significant differences were noted. Though a rough correlation between cholinesterase activities and abnormalities of the electroencephalogram was observed the extent of the fall in the cholinesterase activity is not sufficient to explain the degree of the abnormality in the electroencephalogram.

Hemoglobin iron as a stimulus for the production of ferritin. JOHN K. HAMPTON, JR. (introduced by H. S. MAYERSON). *Dept. of Physiology, School of Medicine Tulane Univ., New Orleans, La.* Studies by Granick and others have shown that iron is stored chiefly in the liver, spleen and bone marrow as the protein-iron compound, ferritin. Evidence is also available to indicate the presence of ferritin in crystallizable amounts in the kidneys of the cat, dog, and human. Its presence in horse kidney has been demonstrated in minute amounts. The present experiments extend these findings to the mouse and rabbit kidney. It has proved difficult to crystallize ferritin from the kidney by the usual $CdSO_4$ method from animals on the usual laboratory regime or after minimal doses of hemoglobin. However, when increasing amounts of hemoglobin are injected intraperitoneally, correspondingly larger amounts of ferritin appear in the mouse and rabbit kidneys. With optimal doses of hemoglobin iron, all animals show the presence of ferritin in their kidneys. More ferritin is found in the liver than in the kidney when small doses of Hb are used, but when larger (and optimal) doses are used, the amounts of ferritin in the liver and kidney tend to be similar. Experiments in progress are designed to investigate these relationships in the human kidney.

Effect of stimulation of sympathetic nerves on capillary filtration. ESTHER HARDENBERGH and JAMES V. MALONEY, JR. (introduced by WILLIAM H. FORBES) *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.* Variations in lymph flow and composition have been used in studying the effects of stimulation of sympathetic nerves on capillary filtration in the head and hind limb regions of the dog. After cannulation of the regional lymphatic vessels, control collections were made during standardized passive movement of the part. The effectiveness of sympathetic nerve stimulation was indicated by skin temperature changes. The quantity of lymph was increased 50–100% over control values during stimulation, while the protein concentration was somewhat reduced. In the hind paw most of the increase occurred in the first 10 minutes of stimulation. Such an increase appears surprising since the filtration pressure should be decreased by the intense precapillary vasoconstriction produced by the stimulation. Several explanations have been considered: *1*) a lymph reservoir which is somehow 'squeezed out' is rendered unlikely by the fact that there is no post-stimulation decrease in flow; *2*) increased post-capillary resistance is rendered unlikely by the fact that lymph flow is increased even when capillary pressures have already been raised by venous obstruction; *3*) gross changes in capillary bed due to closing of arterio-venous anastomoses does not affect the result, since the increase in lymph flow occurs when the paw is immersed in a water bath at 15 degrees Centigrade. There appears to be an increase in capillary filtration during stimulation of sympathetic nerves, but the exact mechanism is not extablished.

Renal excretion of creatinine in man. KENDRICK HARE, HAROLD GOLDSTEIN (by invitation), HENRY L. BARNETT (by invitation), HELEN MCNAMARA (by invitation), and RUTH S. HARE (by invitation). *Dept. of Pediatrics, The New York Hospital and Cornell Univ. Medical College, New York City.* Using the adsorption-elution method of Hare and Hare for creatinine determinations, we have compared the renal plasma clearance of endogenous creatinine with simultaneous inulin clearances in 24 observations on 22 normal subjects ranging from 1 week to 40 years of age. Each observation included 3–9 clearance periods of 10–20 minutes duration. The average creatinine/inulin clearance ratio was 1.03 with a range of 0.82–1.26. The clearance of exogenous creatinine, given by mouth or by vein, was similarly studied in 9 experiments on 6 normal subjects for a total of 76 periods. The average creatinine/inulin clearance ratio was 1.08 with a range of 0.97–1.20. In chronic renal insufficiency the clearance of both endogenous and exogenous creatinine is elevated above the inulin clearance to give creatinine/inulin clearance ratios of 1.29–2.64.

Determination of creatinine in blood and urine. RUTH S. HARE (by invitation) and Kendrick Hare. *Dept. of Pediatrics, The New York Hospital and Cornell Univ. Medical College, New York City.* Pure creatinine solutions of 0.05–0.40 mg. % develop maximal color with alkaline picrate within 20 minutes and remain stable; tungstic, picric, trichloracetic acid or cadmium, zinc or iron filtrates of serum or plasma continue to develop color, as measured at 500 mμ in a Beckman spectrophotometer, for 2 hours or more. The interference of the non-creatinine chromogens can be avoided by adsorbing the creatinine on Lloyd's reagent and eluting with alkaline picrate solution (Borsook, *J. Biol. Chem.* 110:481, 1935). This procedure gives 98–101% recovery of creatinine added to serum or urine; synthetic creatinine, containing radioactive carbon, is completely adsorbed by Lloyd's reagent from urine and from plasma filtrates. Approximately 80% of the material in normal human serum giving the Jaffe reaction is creatinine; 90–100% of the urinary chromogen is creatinine. The serum creatinine falls from 0.8 mg. % in the first week of life to 0.3 mg. % at one month, begins to rise at two years and reaches the adult level of 0.8 mg. % at 15–18 years. In chronic renal insufficiency, the serum creatinine constitutes 90% or more of the total chromogen.

Effects of lanatoside C upon ectopic ventricular activity following coronary occlusion. A. SIDNEY HARRIS and ROBERT H. KOKERNOT (by invitation). *Baylor Univ. College of Medicine, Houston, Texas.* The threshold intravenous dose of lanatoside C for the production of ventricular ectopic discharges in unoperated dogs anesthetized with morphine and light barbital sodium was found to be about 0.125 mg/kg. when administered in two equal parts separated by one hour. The anterior descending arteries were ligated near their origins. The ectopic rhythms produced by coronary occlusion were present during the period from about 8 hours to 3 days following the operation. Lanatoside C in varied doses was given at intervals ranging from 10 hours to 13 days after occlusion. When given during the first 4 days, the ectopic threshold dose, either in the presence or absence of a preexisting ectopic rhythm, was found to be lowered to 0.1 mg/kg. or slightly less, i.e., about 70 to 80% of the control threshold. After 4 days the ectopic threshold was not lower than the preoperative control except in four dogs which at the beginning of the test exhibited electrocardiographic patterns interpreted as aberrant conduction. Tests with 0.06 mg/kg. produced no ectopic discharges at any time. Evidence from other sources indicates that this dose is more than sufficient to increase work output in a failing heart; therefore these findings may indicate that danger associated with ventricular ectopic activity following coronary occlusion is not increased by conservative use of lanatoside C. The dose required to produce retching and vomiting was consistently lower than that which produced ectopic beats in unanesthetized dogs.

Late development of ectopic ventricular activity following coronary occlusion. A. SIDNEY HARRIS and ROBERT H. KOKERNOT (by invitation). *Dept. of Physiology, Baylor Univ. College of Medicine, Houston, Texas.* The anterior descending arteries of the hearts of 25 dogs were ligated proximal to any large branch. The dogs were anesthetized with morphine and a minimal dose of barbital sodium (180 mg/kg.). Under such anesthesia the hearts are fully susceptible to the development of ventricular ectopic systoles. To prevent the occurrence of rapid ectopic discharges and death via ventricular fibrillation within the first 10 minutes following occlusion, the occlusion was made in 2 stages. Partial occlusion was followed 30 minutes later by total obstruction. No animals so operated were lost during the early period in which, following one-stage occlusion, the usual mortality is near 50%. Frequent electrocardiographic observations and records were made. Ventricular ectopic systoles began to appear in significant numbers after periods of 6 to 12 hours in the different experiments. Eight hours was the most common time for the beginning of the rise of ectopic activity beyond an occasional premature beat. At the end of 12 hours ectopic activity was prominent in every animal studied through the period except one that maintained a sinus rhythm. One animal had a moderate ectopic frequency from the time of occlusion. Typically, a maximal ventricular tachycardia was reached between 10 and 21 hours, and ectopic activity persisted from 2 to 4 days. Large highly necrotic gross infarcts were found in almost all hearts upon sacrifice (10 to 22 days).

The uptake of radiocalcium by the skeleton. HAROLD E. HARRISON and HELEN C. HARRISON (introduced by NATHAN W. SHOCK). *Dept. of Pediatrics, The Johns Hopkins Univ. Medical School, and Pediatric Division, Baltimore City Hospitals, Baltimore, Md.* The equilibrium between skeletal calcium and calcium of the body fluids was studied by determinations of the uptake of Ca^{45} by the bone of animals given $Ca^{45}Cl_2$ solution. Groups of rachitic rats, rachitic rats treated with vitamin D_2, and control rats were used. During the experimental period, some of the animals received a high calcium diet and others, a diet low in calcium. The animals were sacrificed at intervals from 2 hours to 2 weeks following administration of Ca^{45}. Measurements of the radioactivity of the calcium of blood serum and the skeleton were made, and the

ratio of Ca^{45} to Ca^{40} expressed in terms of counts per second per mgm. calcium. Equilibrium between body fluid calcium and bone calcium occurs rapidly and in some instances is reached by 72 hours. Epiphyseal bone calcium attains equilibrium with serum calcium more rapidly than that of diaphyseal bone, and the rate of equilibrium is not related to the rate of bone salt deposition. The uptake of Ca^{45} by the skeleton is not a measure of new bone salt addition. Following introduction of Ca^{45} into the body fluids exchange of Ca^{40} of the skeleton for Ca^{45} of the body fluids occurs until the ratio of these isotopes is the same in both phases.

Effect of cinchophen on the secretory activity of Brunner's glands. K. HARTIALA (by invitation) and M. I. GROSSMAN. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Experiments were carried out to determine whether cinchophen when given in doses known to produce ulcers in the dogs has any effect on the Brunner's gland secretion. The juice was obtained either from pouches or everted flaps made from the first part of the duodenum. The original blood supply and nerve connections were maintained. 200 mg. of cinchophen per kg. of body weight was given orally daily for 4 days to 6 dogs. It was noted that the basal secretion was markedly affected after the cinchophen administration. The alterations took place usually after the second day and in some cases even in the first day after the ingestion of drug. The total output of juice diminished to less than a half of the volume of that of the controls. Both the alkali content and the pH were decreased although the great decrease in the total alkali output was mostly a result of the decreased amount of juice secreted. The mucous content was not analysed but in the cases most affected by cinchophen the juice became thinner and was waterish. There was also a decrease in the volume of juice secreted in response to a standard test meal (Pard, milk, white bread).

Inhibition of activity of visual receptors by illuminating nearby retinal areas in the *Limulus* eye. H. K. HARTLINE. *Dept. of Biophysics, Johns Hopkins Univ., Baltimore, Md. and the Johnson Research Foundation, Univ. of Pennsylvania, Philadelphia, Penna.* A discharge of impulses in a given single optic nerve fiber from the eye of *Limulus* can be elicited only by the illumination of some one particular facet of the eye, containing the receptor that gives rise to that nerve fiber. It has been found that illumination of neighboring areas of the eye, while unable to initiate activity in a given fiber, can nevertheless affect the responses to illumination of its receptor. A steady discharge of impulses was obtained by a small spot of light confined to the appropriate facet; illuminating nearby facets then caused a slowing of the discharge. The frequency dropped markedly at first (after a latency of .2–.4 sec.), then rose somewhat; on turning off the adjacent illumination, the frequency increased and within .4 sec. had recovered its original value. This inhibitory effect was greater the higher the intensity and the greater the area illuminated; it decreased with increasing distance between the particular facet and the neighboring area illuminated. The role of this effect in enhancing visual contrast is obvious: brightly illuminated areas inhibit the activity from dimly lighted regions more than the latter inhibit the activity from the former. In *Limulus*, axons of the receptor cells enter a nonganglionic plexus before leaving the eye via the optic nerve; by recording from fibers between the receptors and the plexus, it could be shown that the inhibitory effect of adjacent illumination depended on the integrity of the nervous connection between the receptor and the plexus.

Effects of experimental anaemia on the circulation in dogs. J. D. HATCHER (by invitation), F. SUNAHARA (by invitation), J. C. VAN NOORDWIJK (by invitation) and O. G. EDHOLM. *Dept. of Physiology, Univ. of Western Ontario, London, Canada.* The cardiac output, peripheral blood flow, and blood volume have been measured before, during and after the development of anaemia by small daily haemorrhages. While the haemoglobin level is falling, there is a slight decrease in cardiac output and right auricular pressure. This is considered to correspond to the hypodynamic phase of Howarth and Sharpey-Schafer. When the haemoglobin level falls to 50% or less of the initial value, the cardiac output and the right auricular pressure increase abruptly. At this stage, the daily haemorrhages are stopped; the cardiac output rises to reach a peak value averaging 160% of the pre-haemorrhage outputs. The greatest cardiac output is obtained approximately 2–4 days after the cessation of haemorrhage, at a time when the haemoglobin value is still at its lowest level. The hyperdynamic phase persists for 2–5 days and then the cardiac output declines reaching a normal level at a time when the haemoglobin level has recovered to 75–500% of the pre-haemorrhage value. Oxygen consumption is increased significantly during the hyperdynamic phase. The saturation of the mixed venous blood diminishes during the hypodynamic phase and rises in the hyperdynamic phase. The total blood volume decreases approximately 25% during the period of haemorrhage, while the plasma volume remains relatively unchanged. The plasma volume and total blood volume rise during the hyperdynamic phase, but the overall changes are not marked.

Consciousness and reflex potentialities of dogs during immersion hypothermia. H. O. HATERIUS

and A. H. HEGNAUER. *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Random observations on CNS activity on more than 150 dogs during immersion hypothermia are worthy of record. The choice of pre-cooling anesthetic is of paramount importance. Light ether or cyclopropane, withdrawn periodically, reveals with considerable accuracy the rectal temperature (R.T.) at which consciousness is abolished by cold narcosis. This varies from 22.5° to 26° R.T. (24.5° to 28° cerebral temperature). The 22.5° observation was made during rewarming, at which point the dog righted his head and attempted standing. Failure to stand was due to intense shivering and limb spasticity. In one experiment after withdrawal of cyclopropane the dog strained at his bonds at R.T. 20.5 and again at 15.8 (cerebral temperatures 23.5° and 20.5° resp.). Spontaneous reflex activity was noted as follows: shivering, which under pentothal-Na is rarely observed below 21°, may under light ether be evident at 16° or 17°; vocalization (whimpering, whining, moaning) to 17°, barking in one case at 20°; spontaneous jaw, head or limb movements to 17° (exceptional case 15.8°); spontaneous respiration in one preparation at 11.8° (2/min.), in 2 preparations below 13.6°, and a total of 12 dogs breathed at R. T. 16° or lower (cerebral temperature 20° or less). After pentothal alone consciousness during rewarming is never observed at rectal temperatures below the thirties, although the last injection may have been made some 2 or 3 hours earlier. Furthermore, the temperatures at which the above reflexes disappear are all higher than those recorded.

Ascorbic acid metabolism in patients with certain gingival disturbances. RAYMOND HAYES (by invitation), WALTER M. BOOKER and MARIANNA B. SEWELL (by invitation). *Dept. of Oral Medicine, College of Dentistry, and Department of Pharmacology, College of Medicine, Howard Univ., Washington, D. C.* Fasting plasma ascorbic acid in patients suffering with various types of gingivitis was found to be significantly lower than in a control normal group of patients. The cell ascorbic acid levels of the blood in the same patients was equal to or in some instances greater than that of the control groups. By and large, only when there is deficiency in both plasma and cell ascorbic acid were clinical signs of gingivitis striking or even present. When ascorbic acid is administered (orally or intravenously) to patients and to normal subjects, the plasma level rises faster than the cell level, suggesting something of a barrier between the plasma and the cells. Many of the patients excrete ascorbic acid in the urine in greater quantity and at faster rate than the normal subjects. Efforts are in progress to compare the rate of urinary excretion with the clinical state of the patient regarding gingivitis. There are presented some outstanding examples of extremely low kidney threshold regarding ascorbic acid excretion among some of the patients. The low kidney threshold to ascorbic acid apparently bears no relationship to the general nutritional state of the patient in terms of plasma proteins and blood sugar. Diminution of serum chlorides upon the intravenous or oral administration of 100 to 300 mg. of ascorbic acid is seen in all patients and in normal subjects.

Effect of pituitary adrenocorticotropin on the plasma hypertensinogen concentration of dogs. F. W. HAYNES, P. H. FORSHAM (by invitation), G. W. THORN and L. DEXTER (by invitation). *Dept. of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.* The effect of purified hog anterior pituitary adrenocorticotropin (ACTH, Armour) on plasma hypertensinogen was studied in trained, unanesthetized dogs. ACTH, without appreciable contamination with thyrotropic, gonadotropic and growth hormones, but containing posterior pituitary activity up to 0.08 units per mg., was used in daily doses equivalent to 20 to 80 mg. of Armour standard (LA-1-A). Hypertensinogen was determined by incubation of plasma with an excess of hog renin and cat assay of the hypertensin formed. Data are based on the mean of at least 4 satisfactory assays on each plasma sample. In 5 experiments on normal dogs receiving ACTH, either intramuscularly in divided doses over a 3-day period or intravenously as an infusion over several hours, the hypertensinogen concentration increased for several days and then returned to its previous level. The control hypertensinogen levels of these dogs (1.7–3.1 cat units per cc. of plasma) were increased by 0.6 to 2.9 cat units after ACTH. Adrenocortical activation was suggested by a significant fall in eosinophils during ACTH administration. ACTH failed to influence the hypertensinogen concentration in an adrenalectomized dog. Hypertensinogen and eosinophil levels failed to show a significant change in control experiments designed to test the effect of sampling and of posterior pituitary contamination in a normal dog.

Corticosteroid release from the isolated adrenal gland. OSCAR HECHTER. *Worcester Foundation for Exptl. Biology, Shrewsbury, Mass.* Isolated beef and sheep adrenal glands were perfused with homologous citrated blood. Following the addition of adrenocorticotrophic hormone (ACTH) it was consistently observed that the venous blood contains an increased amount of formaldehydegenic substances (F.S.) in the neutral chloroform fraction designed to concentrate adrenocortical steroids. These F.S. may be obtained from perfusates by extraction of the acidified blood with chloroform or

chloroform-ethanol mixtures or by chloroform extraction of dialysates of blood. With both methods, it has been found that there is either slight or no release of F.S. from glands perfused in the absence of ACTH. In 5 experiments with beef glands, following the addition of ACTH (20 to 200 μg per cc.) there is an average release of F.S. corresponding to 26 μg (Range 10–42) equivalents of 11-desoxycorticosterone per minute per gland (weights 15–20 gm.). In some glands, increased F.S. output was maintained for as long as 4 hours. In 2 experiments with sheep glands the release of F.S. following ACTH was of the same order of magnitude per gm. gland. Assay of fractions, from four different beef adrenal persates for glycogenic activity, using the method of Olson *et al.* (*Endocrinology* 35: 430, 1944) revealed two to be active; 50 and 100 μg F.S. equivalent to 50 and 60 μg. Compd E. Kendall respectively. The other two extracts were inactive at a level of 100 and 400 μg F.S. In 4 experiments, despite wide variations in dosage, the intrarterial injection of epinephrine consistently failed to release F.S. from perfused glands. The significance of these *in vitro* findings will be discussed.

Temperature gradients in dogs in immersion hypothermia. A. H. HEGNAUER. *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* The procedure followed in this laboratory for producing rapid, drastic hypothermia in dogs (after preliminary anesthetization and required surgery—animals in supine position on dog board) is immersion to the neck in an iced bath at 2°–4° C. With the head end of the board a few inches higher than the foot the portion of the animal unimmersed includes the head, the ventral third of the thorax and all but the nape of the neck. The proportion of exposed to submerged body is approximately that obtaining when a man floats on water in a 'Mae West' life vest. Under these conditions there occur not only core to surface, but also cerebral (C.T.) to rectal temperature (R.T.) gradients. Cerebral, mediastinal (M.T.), right heart blood (B.T.) and rectal temperature as well as muscle and skin temperature (or some combination of these) is recorded continuously via Speedomax (Leeds & Northrup). Plotting R.T. linearly as abscissa and deviations therefrom as ordinate, curves are obtained from which the following conclusions may be drawn: *1*) C.T. is higher than R.T., and progressively so with decreasing R.T. *2*) M.T., initially slightly below rectal, is equal to the latter at R.T. 36° and subsequently parallels the curve for C.T., about 1° lower. *3*) Right heart blood (B.T.) is initially ½° less than R.T., falls further on immersion and then crosses R.T. when latter reaches 28°, and thence parallels C.T. and M.T. When R.T. reaches 12° the C.T., M.T., and B.T. are 18°, 17°, and 16° respectively. Skin temperature from submerged area rapidly approaches bath temperature. Muscle temperature (hind leg) cools more slowly than skin, but much more rapidly than R.T.

Pulse rate and blood pressure of dogs in immersion hypothermia. A. H. HEGNAUER and H. O. HATERIUS. *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Average pulse rate (P.R.) and blood pressure of 18 lightly anesthetized dogs (sodium pentothal) obtained during cooling in an iced bath (2°–4° C.) were plotted against the right heart blood temperature (B.T.). A thermocouple was placed in right heart with continuous recording on Speedomax in degrees centigrade. The pre-immersion P.R. was 155. Disregarding the initial abrupt increase and decrease in rate from moment of immersion to B.T. 36° the rate follows a smooth regression curve to B.T. 15° at which point the P.R. is 15. Between B.T. 36° and 26° the apparent Q_{10} = 2.1, and increases to 5.5 between 25° and 15°. Plotting log P.R. vs 1/T° abs. revealed no sharp breaks. Tentatively as a working hypothesis a regression curve (Q_{10} = 2.5) was constructed, passing through the origin of the experimental curve (at P.R. 155). The computed curve again coincides with the P.R. curve at B.T. 19°, when the two follow a similar course. Between B.T. 39° and 19° the P.R. curves lies well above the computed curve, and it is suggested the elevation is a measure of sympathetic activity at these temperatures.

Action of heparin on protoplasmic gelation. L. V. HEILBRUNN. *Dept. of Zoology, Univ. of Pennsylvania, Philadelphia, Penna.* Earlier studies from this laboratory have indicated that the gelation of protoplasm is similar to the clotting of blood. Before a cell divides, its protoplasm gels and the appearance of the mitotic spindle is preceded by the so-called mitotic gelation. In the egg of the worm *Chaetopterus*, this mitotic gelation is prevented by immersion of the eggs in dilute solutions of heparin. Suppression of mitotic gelation results in an inhibition of cleavage. Similar results were obtained with frog eggs. The initiation of mitosis in these eggs tends to be prevented by the presence of heparin. There is an indication, therefore, that in living cells the colloidal behavior of the protoplasm is to some extent at least determined by the equilibrium between thrombin-like substances and heparin or heparin-like substances. Actually, in the *Chaetopterus* egg there is direct evidence for the presence of either heparin or some substance which stains like it.

The role of the Sertoli cell in the testis-pituitary axis in men. CARL G. HELLER, WILLIAM O. MADDOCK (by invitation), EDWIN C. JUNGCK (by invitation), and WARREN O. NELSON. *Depts. of Medicine and Physiology, Univ. of Oregon Med.*

School, Portland, Oregon, and Dept. of Anatomy, Univ. of Iowa Med. School, Iowa City, Ia. The supporting cell of Sertoli is claimed to elaborate a non-androgenic hormone whose role is to inhibit the secretion of pituitary gonadotrophin. If this is correct, no alterations in gonadotrophic output should occur in instances in which Sertoli cells remain intact and germinal cells disappear. Testicular biopsies in men with azoospermia or oligospermia have revealed 34 cases in which at least some of the seminiferous tubules were devoid of all germinal elements except the Sertoli cell. Such tubules constituted 10% to 100% of the total seminiferous tubules and were associated with either hyalinization of the basement membrane or with tubules having germinal cells present, or both. Despite the intact Sertoli cells, elevation above normal in the gonadotrophin titers was regularly encountered in cases having less than 70% of active tubules. A roughly direct proportionality exists between the height of the titers and the degree of spermatogenic failure. This suggests that the Sertoli cell does not enter into testis-pituitary regulation, but suggests that during active spermatogenesis, gonadotrophins are inactivated: in the presence of incomplete or failing spermatogenesis, a smaller amount of gonadotrophin is utilized, allowing for a rise in titer.

Adrenal cortical function and the effect of adrenocorticotropin in myasthenia gravis. LEON HELLMAN (introduced by R. E. WESTON). *Medical Division, Montefiore Hospital, New York City.* Hypertrophy of the lymphoid tissues and the thymus occurs in many patients with myasthenia gravis and in patients and animals with adrenal cortical hypofunction. Atrophy of the thymus can be induced in animals by the administration of adrenocorticotropin (ACTH). Since surgical removal of the usually enlarged thymus appears to benefit patients with myasthenia gravis, ACTH was administered to patients with myasthenia gravis in an attempt to 'hormonally' remove their thymuses. Several adrenal cortical functions were studied in these patients. The subjects were 5 patients with classical myasthenia gravis, responsive to prostigmine, none of whom had demonstrable thymic tumors. Injection of 35 mg. ACTH (Armour) produced the responses characteristic of normal adrenal function. Circulating eosinophils fell over 60%, circulating lymphocytes fell over 40%, and uric acid-creatinine ratio increased over 70%. Sodium restriction to 100 mg. daily for 10 days did not produce adverse effects or a tendency to lose sodium. 17-ketosteroids were normal in 3 of the patients tested. 0.2 mg. epinephrine produced a 60% fall in circulating eosinophils. Administration of 125 mg. ACTH daily for 4 days to 3 patients produced the changes associated with adrenal cortical hyperfunction. If anything, the patients' clinical courses were adversely affected. This may be related to the potassium diuresis or the altered intracellular-extracellular Na-K ratio produced by ACTH. Administration of 500 mg. synthetic Compound A (supplied by Merck and Company) to 2 patients produced a temporary dramatic restoration of muscular function.

Effect of adrenocorticotropin in human chronic lymphatic leukemia. LEON HELLMAN (introduced by R. E. WESTON). *Medical Division, Montefiore Hospital, New York City.* The administration of adrenocorticotropin (ACTH) produced a decrease in circulating lymphocytes and eosinophils in the human and adrenal cortical extracts are said to produce a lymphopenia and regression of lymphoid tissue in mice with spontaneous lymphatic leukemia proved by marrow aspiration were injected with 25–125 mg. ACTH (Armour). The initial peripheral white count ranged from 3000–400,000 WBC, the majority being in the upper range, and the lymphocytes were usually over 90% of the total WBC. After ACTH, no leukemic patient showed a significant decrease in circulating lymphocytes, but all except 2 showed a fall in circulating eosinophils of over 60%. Initial eosinophil counts tended to be low. Total WBC remained unchanged while the uric acid-creatinine ratio was usually increased over 60%. No changes were observed in lymph nodes removed from two patients before and after the ACTH. In contrast, 25 normal controls given simultaneous injections of equal amounts of the same ACTH, showed a fall of over 40% in circulating lymphocytes, a decrease of over 60% in circulating eosinophils, an increase in total WBC of 50–120%, and an absolute polymorphonuclear leukocytosis with a shift to the left. 25 cc. lipoadrenal cortex extract (supplied by the Upjohn Company) produced changes in normals and leukemics similar to ACTH. The effect of ACTH on the peripheral blood may be useful as a biologic test for lymphoid leukemia and has been used successfully in two patients with aplastic anemia. It is suggested that mouse lymphoid leukemia differs significantly from the human variety.

The concurrent feeding of amino acids. RICHARD HENDERSON (by invitation) and ROBERT S. HARRIS. *Nutritional Biochemistry Labys., Massachusetts Inst. of Technology, Cambridge, Mass.* Groups of weanling rats were fed a basal diet containing casein (30), corn starch (55.4), hydrogenated fat (10), tryptophan (0.17), histidine (0.13), saltmixture (4) and vitamin mixtures (0.36). Control groups received the basal diet + 1% lysine. Test groups received the basal diet and the basal diet + 2% lysine at alternate feedings. The lysine replaced equal weights of corn starch in the diet. Groups weer fed (1) one hour and fasted 7

hours, (2) one hour and fasted 5 hours and (3) one hour and fasted 3 hours throughout successive experiments lasting 27 days. A time-interval feeder was devised for the automatic opening and closing of the feeding jars. Significant differences were noted in nitrogen retention and in bodyweight gain, weight gain per gram of diet eaten, weight gain per gram of lysine eaten. These differences were greater as the feeding intervals lengthened. Thus a delay of three hours or longer, in the feeding of lysine as a supplement to a lysine-low diet interfered with metabolism. Lysine must be fed concurrently with other amino acids in the diet for most efficient utilization by the rat.

Factors determining cerebral oxygen supply during positive acceleration. J. P. HENRY, O. GAUER (by invitation), E. E. MARTIN (by invitation), S. S. KETY (by invitation) and K. KRAMER (by invitation). *Aero Medical Laby., Air Material Command, Wright-Patterson Air Force Base, Dayton, Ohio.* Extraordinarily low mean cephalic arterial pressures of less than 20–30 mm. Hg (blackout) can be endured during positive accelerations lasting as long as 3 minutes without loss of consciousness. When compared with the effects of a comparable drop in pressure during hemorrhagic shock, there is an unexpected retention of mental performance and orientation. In order more clearly to define the mechanisms involved, radial arterial and cerebral venous pressures, and arterial and cerebral venous oxygen saturations were measured in man during acceleration. These parameters were measured two at a time using electrically operated gauges for the pressures and a Kramer glass cuvette oximeter for the oxygen saturations. The fall in arterial pressure at head level induced by the acceleration was closely followed by a drop in jugular venous pressure. In spite of black-out (4.5 *g*) one subject still had 60 mm. Hg mean pressure differential across the brain. The venous pressure became less negative as arterial pressure rose during the cardiovascular response to the sharp fall in arterial pressure induced in the carotid sinus by the acceleration. Arterial oxygen saturation also started to fall approximately 7 seconds after acceleration commenced and by the end of the 15 second run at 4.5 *g* it had attained approximately 90%. Further, after a 5–7 second delay the venous oxygen saturation fell and showed fluctuations paralleling the arterial and venous pressure changes. Typical changes in venous oxygen content were from 10.5 vols. % resting to 7.5 vols. % at blackout. Thus arterial as well as venous oxygen content should be known to determine the oxygen supply of the brain during prolonged acceleration. The extent of the fall in cerebral venous oxygen content at blackout points to an inadequacy of cerebral oxygen supply.

Some responses of man to internal thermal stimulation. AUSTIN HENSCHEL, HENRY LONGSTREET TAYLOR and ANCEL KEYS. *Laby. of Physiological Hygiene, Univ. of Minnesota, Minneapolis, Minn.* The gastric motility, blood sugar concentration, blood pressure, pulse rate and finger skin temperature responses to internal thermal stimulation were measured on a total of 16 young men who were free from signs of gastrointestinal and cardiovascular abnormalities. The thermal stimulus was a standard ice cream mixture to which 15% barium sulphate was added. Either 400 or 100 gm. portions of the test meal was eaten in exactly 5 minutes; the test meal temperature was −8°C., 26°C. or 65°C. Gastric motility as measured by gastric emptying time was not altered by the temperature of the test meal. The blood sugar concentration increased a maximum of 22 mg. % 30 minutes after the −80°C. test meal; the maximum after the 65°C. test meal was 12 mg. %. Finger skin temperature decreased an average of 3.25°C. at 10 minutes after the −8°C. test meal and was not back to normal at 50 minutes; the 26°C. and 65°C. test meals had no effect. An increase of 14.8 mm. Hg systolic and 17.6 mm. Hg diastolic blood pressure and 20.2 beats per minute pulse rate occurred during ingestion of the 400 gram test meal at −8°C. The values were normal within 2 minutes after completion of the meal. There was no blood pressure, pulse rate or skin temperature response to the 100 gram test meal served at −8°C.

Thermistors as applied to temperature measurements in animals. J. F. HERRICK AND E. GLARBORG. *The Mayo Foundation, Rochester, Minn.* The thermistor (thermally sensitive resistor) has proven a convenient, simple, rugged and accurate method for measuring the temperature of various animal tissues in our laboratory. This type of thermometer introduces a new order of sensitivity so far as resistance thermometers are concerned. We are using thermistors for measuring temperatures as low as −40°C. in certain investigations on frost-bite and also temperatures as high as animal tissues can be heated without irreversible changes being produced. The thermistor is particularly suitable for measuring temperature changes in bone and bone marrow. Rectal temperatures of small animals can be recorded continuously quite conveniently. The thermistor can be introduced easily into arteries, veins, muscle and subcutaneous tissue and left in place throughout the day when studying changes in temperature caused by various methods of heating or cooling. We have been using the thermistor for measuring changes in skin temperatures of the dog for almost two years and find it a very convenient method. All temperatures may be recorded on moving photographic film or by

direct observation. Temperatures may also be observed at a considerable distance from the site of measurement. The various types of thermostors, the necessary equipment for measuring temperatures and the method of calibration will be described, and representative data discussed.

Mechanism and management of edema. GEORGE R. HERRMANN, JOHN W. CHRISS, E. H. SCHWAB and PAUL M. SIMS. *Univ. of Texas Medical School, Galveston, Texas.* The newer conceptions of cardiac output and renal blood flow in the genesis of sodium retention and edema formation are discussed. The influence of mercurials on sodium output is stressed. Thiomerin—an organic mercurial with a sulfhydril radical and a new diuretic with low toxicity—is discussed. Satisfactory diuresis has been obtained following both subcutaneous and intravenous injections. Thiomerin given intravenously has also been studied for any significant electrocardiographic changes.

Myocardial insufficiency and its management with especial reference to a new and safe mercurial diuretic. GEORGE R. HERRMANN, JOHN W. CHRISS, E. H. SCHWAB, MILTON R. HEJTMANCIK and PAUL M. SIMS. *Univ. of Texas Medical School, Galveston, Texas.* Sodium retention is the most important factor in edema formation in congestive heart failure. Decreased cardiac output results in diminished renal circulation, with 90-fold sodium reabsorption. Sodium, with its retained water added to the extracellular fluids, produces edema. Organic mercurial decrease tubular reabsorption of electrolytes and water, producing diuresis. A 70-fold sodium excretion accompanies mercurial diuresis. Intravenous mercurial diuretics have resulted in sudden death, with ventricular fibrillation demonstrated in 2 cases, and 20% of patients show cardiac mechanism disorders. In attempting to counteract this myocardial toxicity, a sulfhydril group was substituted for theophylline. This mercapto, thiomerin, is less toxic than any other mercurial diuretic. Thiomerin is well tolerated subcutaneously, and produces satisfactory diuresis. Intravenous use to compare its effects on the heart with that of other mercurials has resulted in a minimum of even slight reactions. This drug is potent, less irritating, and practical to use.

An estimation of the regional distribution of the cutaneous heat losses. ALRICK B. HERTZMAN AND WALTER C. RANDALL. *Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

We have explored the possibility of calculating the total heat losses from the surface of the body and their regional distribution from the estimations of cutaneous blood flows (photoelectrically recorded skin pulses) and the A–V temperature differences (oral temperature minus skin temperature) in the various skin regions. Total heat losses thus estimated approximate those which are calculated from O_2 consumptions and changes in average body temperature. The corresponding fractions of the total heat losses as contributed by the various skin regions of the resting nude subject are approximately: trunk 25, leg 19.5, arm 14.2, face 15.5, hand 15.2, foot 10.7%. These fractions vary from subject to subject, with the metabolic rate and environmental conditions. The various skin regions may be grouped (in resting nude subjects) into two divisions: one group (face, palm, plantar surface and digit pads) characterized by high rates of blood flow (above 0.06 cc/cm²/min.) and high rates of heat loss (above 0.2 calories/cm²/min.); the other group (trunk, leg, arm and dorsal surfaces of hand and foot) characterized by low rates of blood flow (below 0.03 cc/cm²/min.) and low rates of heat loss (below 0.08 calories/cm²/min.). The value of the fraction, blood flow divided by the difference in temperatures of skin and air, shows a similar regional distribution, suggesting that differences in evaporative rates may set flow levels in the corresponding skin regions.

Determination of acetylcholine in cell-free enzyme systems by a colorimetric method. SHLOMO HESTRIN and EMILY F. HEDAL (introduced by DAVID NACHMANSOHN). *Dept. of Neurology, College of Physicians & Surgeons, Columbia Univ., New York City.* The development of a chemical micro-method for acetylcholine has permitted assay of this ester in the presence of choline and acetate in large excess. The method utilizes the fact that esters are rapidly converted at alkaline pH by hydroxylamine into corresponding hydroxamic acids. The latter may be determined colorimetrically as in the procedure for acyl assay which has been described by Lipmann and Tuttle.

Results obtained with the use of this method in experiments on synthesis of acetylcholine by acetylcholine-esterase and choline acetylase will be discussed.

The antagonism of cinchona alkaloids to the circulatory effects of intravenous epinephrin. EDWIN P. HIATT. *Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N. C.* The effect of standard intravenous doses of epinephrin (0.5 cc. of 1:10,000) on arterial blood pressures of unanesthetized dogs was recorded photographically using a membrane manometer. The dogs were then given several oral doses of quinine or quinidine sulfate (15 mg/kg. at intervals of several hrs.) attaining a sustained plasma concentration of 5 to 10 mg/l. These drugs abolished both the pressor effect and the changes in rate which usually follow epinephrin injection. Lower concentrations of the alkaloids had less effect and larger doses of epinephrin overcame the inhibitory effect of the alkaloids. These results confirm the effects reported

repeatedly since 1919 by investigators using intravenous injections of the cinchona alkaloids without measurement of plasma alkaloid concentrations. The reciprocal effect was shown by Dreisback and Hanzlik (*Jour. Pharm. & Exp. Therap.* 83: 167, 1945) and others, using epinephrin to counteract the depressor effect of intravenously injected cinchona alkaloids. We have previously demonstrated that these drugs cause renal vasodilatation and decrease the blood pressure of dogs with experimental neurogenic hypertension. Perhaps these effects are due to an inhibitory effect on adrenergic sympathetic endings. Since it has been demonstrated that these alkaloids can inhibit certain parasympathetic endings as well as motor fibers to skeletal muscles (*Jour. Pharm. & Exp. Therap.* 85: 55, 1945), it may be these agents can inhibit all motor nerve endings.

Effect of prostigmine and urecholine on human gastric motility. N. C. HIGHTOWER, JR. (by invitation), C. F. CODE, F. T. Maher (by invitation) and C. G. MORLOCK (by invitation). *Section on Physiology, Mayo Foundation and Division of Medicine, Mayo Clinic, Rochester, Minn.* Gastric motility was recorded by means of a system consisting of an undistended balloon containing approximately 28 cc. of water and an air cushion of 122 cc. between the water-filled balloon and a glass-spoon pressure recorder. The volume pressure coefficient of the system was 0.13. The pressures developed in the balloon were recorded photographically, 1 cm. of H_2O pressure producing 1 mm. of deflection in the record. Studies were made in normal human beings during fasting, and after the intramuscular injection of 0.5 mg. of prostigmine and after the oral administration of 5 mg. of urecholine. A quantitative analysis of the photographic records has been attempted, and the results of this analysis have been summarized statistically. The data show that the rate of type II contractions (propulsive type) never exceeded the rate of the smaller type I contractions ('20 second rhythm' of Carlson), and that the administration of the drugs in question did not alter this relationship. Neither drug changed significantly the total amount of motility activity in the stomach, nor did they affect the incidence of the various types of motility. The drugs produced a slight increase in the mean height of the type II contractions, but the significance of this change is questioned. The study supports the hypothesis that the small type I contractions represent a basic fundamental rhythmic sequence of motility activity in the stomach.

Effect of tridione on convulsions caused by di-isopropyl fluorophosphate (DFP). H. E. HIMWICH, C. F. ESSIG (by invitation), and J. L. HAMPSON (by invitation). *Army Chemical Center, Medical Division, Edgewood, Md.* In a series of more than 100 experiments di-isopropyl fluorophosphate (DFP) was injected into one common carotid artery of curarized rabbits under artificial respiration. With the small amounts of atropine required to protect the heart from succumbing to excessive acetylcholine, it was possible to give DFP in dosage large enough to produce convulsions and still maintain life. Changes were noted in the electroencephalogram which in their most severe form resembled the condition of status epilepticus. Since the subsequent analysis of the brain tissues disclosed a great fall in the action of cholinesterase, the enzyme which hydrolyzes acetylcholine,the changes in the brain waves were presumably produced by excessive acetylcholine. In 10 experiments the intravenous administration of tridione corrected the grand mal type of electroencephalogram in doses of 0.1 to 0.3 gm/kg. and restored the electroencephalogram to normal with doses of 0.2 to 0.4 gm/kg. In other experiments, in which tridione was injected before DFP, tridione exhibited a preventive action for approximately 4 hours. Though the grand mal electrical pattern was prevented the normal electroencepahlogram was not maintained and increased activity was observed. The administration of tridione in no way influenced the profound fall in cholinesterase activity. It would seem therefore that tridione counteracts the effects of excessive concentrations of acetylcholine in the brain.

Cholinergic nature of the vestibular receptor mechanism; forced circling movements (motion picture). H. E. HIMWICH, C. F. ESSIG (by invitation), J. L. HAMPSON (by invitation), P. D. BALES (by invitation) and A. M. FREEDMAN (by invitation). *Medical Division, Army Chemical Center, Md.* Di-isopropyl fluorophosphate (DFP) was injected into the common carotid artery of animals belonging to four species: Rabbits, cats, dogs and monkeys. Though the dosage required varied with the species, the results were similar. The head turned to the side opposite to that of injection and compulsive circling movements were made to the side opposite, hence the name adversive syndrome. Labyrinthectomy or section of the eighth nerve prevented the development of the syndrome when the injection was made on the operated side but not when the DFP was introduced into the other common carotid artery. Either atropine or scopolamine cured the syndrome.

Ketosis in diabetic humans in response to the stress of interpersonal adjustment. LAWRENCE E. HINKLE, JR. and GEORGE T. CONGER (introduced by STEWART WOLF). *Depts. of Medicine and Psychiatry of the New York Hospital and Cornell Univ. Medical College, New York City.* Eleven diabetic individuals were subjected to experimental study. Increased glycosuria and ketosis

were observed repeatedly during conflicts associated with fear, depression, frustrated anger and, as far as could be ascertained in the absence of significant changes in diet, insulin or activity. Accordingly, 2 of these subjects were studied in the hospital where diet, insulin and activity were carefully controlled. A marked increase in glycosuria in one, and clinically typical keto-acidosis in the other, were induced when the individuals were subjected to significant threats to their emotional security. Three of the subjects were studied in short-term experiments under sodium amytal narcosis. In all a significant rise in the concentration of ketone bodies in the peripheral blood occurred during a discussion of their personal conflicts. Following reassurance, in each case the ketones fell to lower levels. Ketone measurements were made by the micro-method of Greenberg and Lester. Blood samples were also obtained from a catheter introduced directly into the hepatic vein. Significant elevations of ketone content during tension and conflict with return to control levels during reassurance were demonstrated.

Acute renal cortical ischemia produced by stimulation of the pressor area of the cerebral cortex. E. C. Hoff, J. F. Kell, Jr. (by invitation), Nelson Hastings (by invitation), E. H. Gray (by invitation) and D. M. Sholes (by invitation). *Neurological Science Laby. of the Medical College of Virginia, Richmond, Va.* Green and Hoff (*Am. J. Physiol.* 118: 641, 1937) reported in cats and monkeys that limb volume usually increased while kidney volume diminished during pressor responses evoked by electrical stimulation of the cerebral cortex. Denervation of the kidney abolished this renal vasoconstriction so that the kidney passively dilated during the mean blood pressure rise. In the present experiments, acute blood pressure rises, lasting 15 to 90 seconds, were evoked by stimulation, unilaterally, of foci on gyrus proreus, gyrus sigmoideus, and orbital and mesial surfaces of the cat's cerebral cortex. In each experiment, one side only of the cerebral cortex was stimulated repeatedly for 1 to 2 hours using an inductorium or square-wave generator. Finally, during the height of a maintained pressor response, 30 to 50 cc. of India Ink solution were injected into the right common carotid artery towards the aorta. There was pronounced vasoconstriction in the cortex of both kidneys as evidenced by virtual exclusion of the ink from the renal cortical vascular bed. This was in sharp contrast to findings in unstimulated control animals, in which both kidneys were deeply injected throughout. It is concluded that bilateral renal cortical ischemia results from excitation of the pressor area of the cerebral cortex, and that this vasoconstriction is part of the generalized vasomotor response underlying the acute experimental blood pressure rise. Possibly certain abnormal cerebral activity in man may result in persistent or recurrent renal cortical ischemia, and this mechanism may lie at the basis of essential hypertension.

Nature of apneusis and the pontine influence on respiration. Hebbel E. Hoff and C. G. Breckenridge (by invitation). *Dept. of Physiology, Baylor Univ. College of Medicine, Houston, Texas.* Dogs decerebrated by transection after occlusion of the carotids and the basilar artery at the lower border of the pons universally exhibit apneustic breathing, after transection encroaching on the pons and vagotomy. Apneustic breathing, consisting of alternate periods of inspiratory spasm and expiration at slow rates (1 or 2/min. or less), is in the dog capable of maintaining life for several hours. When the carotids are occluded only during transection, pontine section and vagotomy produce apneusis in the majority of experiments, but certain animals show 'normal' respiration. Animals showing apneustic breathing resume 'normal' breathing terminally as the preparation deteriorates. When a further section encroaching on the medulla is made in 'apneustic' animals, the phases of inspiratory spasm are converted into periods of phasic 'normal' inspiration and expiration akin to Biot's breathing, often reverting to 'normal,' or 'normal' breathing appears immediately. Intravenous injection of cyanide produces apneusis or prolongs and intensifies it. When the carotid bodies and sinuses are denervated, apneusis does not appear after pontine decerebration and vagotomy; 'normal' or Biot's breathing alone occurs.

It is concluded that: *a*) the medullary respiratory center can maintain periodic respiration in the absence of the pons and the vagi; *b*) apneusis represents an exaggerated inspiratory response to anoxemic stimulation via the carotid bodies 'occluding' the normal respiratory action of the medullary center; and *c*) such enhancement probably represents unchecked activity of the pontine portion of the midbrain facilitatory center.

Failure of topical calcium and magnesium to prevent eugenol-induced desquamation of gastric mucous epithelium. Franklin Hollander and Shirley D. Kraus (by invitation). *Gastroenterology Research Laby., Mount Sinai Hospital, New York City.* Previous studies have demonstrated that the mucigogue action of topical stimuli on the gastric mucosa of Heidenhain pouch dogs is usually accompanied by extensive desquamation of the surface epithelium, progably because of a loosening of the cement substance. A similar, though less marked, decrease in cellular cohesion may also underlie the invasive character of cancerous tissue, and evidence is accumulating in the literature that Ca- and Mg-ions are of considerable importance for maintaining the integrity of the cell-

binding mechanism in such tissue—as well as in several varieties of normal tissue from lower forms of life. Therefore, the present study was designed to determine whether the shedding of gastric mucous epithelium, induced by 1% aqueous eugenol alone, can be influenced markedly by the addition to this mucigogue-desquamating agent of $CaCl_2$ (0.02, 0.04, and 0.06%), alone or with $MgCl_2$ (0.01 and 0.03%). $NaHCO_3$ was added to adjust the pH to about 7. Mucus specimens, collected in 4 half-hour intervals following stimulation, were examined for viscosity, opacity, and the presence of cells (microscopically). No essential difference in these characteristics was observed between these experiments and controls without inorganic salts. Further experimentation is required to show whether the desquamating effect of eugenol emulsion is entirely independent of Ca- and Mg-ions, even though these may be part of the cement substance, or whether this effect is so powerful, even with 1% concentration, that it completely overcomes the influence of those ions under the conditions of these experiments.

Effect of sympathetic blocking procedures and of sympathectomy on peripheral blood flow. S. W. Hoobler, J. W. Avera, W. J. Little, M. M. Peet and R. C. Bassett (introduced by A. H. Smith). *Depts. of Medicine and Surgery, Univ. of Michigan Hospital, Ann Arbor, Mich.* Certain sympathetic blocking techniques all of which abolished toe-thigh temperature gradients were studied by means of the venous occlusion plethysmograph. Resting blood flow in the foot averaged 0.7 cc/100 cc. limb volume/minute (range 0.5–4.0). Average maximum flow after reflex body heating was 4.5 cc. (range 2-7); after 500 mg. tetraethylammonium chloride intravenously: 7.6 cc. (2.8–10.3); after unilateral paravertebral block (L1–L3): 18 cc. (11–26); after caudal anesthesia (1.5% metycaine to D3) or spinal anesthesia: 34 cc. (16–60); within 12 hours after lumbar sympathectomy in 3 cases: 30.1 cc. (12–36). In the latter instances the early postoperative result corresponded well with preoperative paravertebral block of spinal anesthesia. The disparity between increase in digital temperature and increase in blood flow as determined in the plethysmograph was emphasized by an observation under caudal anesthesia utilizing 0.75% metycaine. In this hypertensive patient skin temperature gradient was abolished and anesthesia to pin prick as well as anhydrosis occurred to a level of D2. The blood pressure did not fall nor was there a measurable increase in blood flow in the foot. It may be concluded that complete block of the appropriate sympathetic ganglia or spinal vasomotor outflow can produce very great increases in foot blood flow, but that total block can not be assumed from abolition of skin temperature gradient or of sudomotor activity alone.

After 3 out of 4 sympathectomies, plethysmographic measurements showed a reduction to preoperative levels in 3 to 7 days. This was maintained thereafter for periods up to 15 months despite continued elevation of the digital temperature and anhydrosis.

Blood temperature in the heart and blood vessels of the dog. Steven M. Horvath, E. L. Foltz (by invitation), A. Rubin (by invitation) and B. K. Hutt (by invitation). *Dept. of Physical Medicine, Graduate School of Medicine and the Dept. of Pharmacology, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* Temperatures of the blood, tissues, skin and rectum in anesthetized dogs were determined. Cournand type catheters with thermocouples at the tip were placed roentgenoscopically in the coronary sinus and the right ventricle or pulmonary artery. Smaller catheters were similarly inserted into the femoral artery and vein. The emf developed by the thermocouple was measured with a type K-2 Leeds and Northrup potentiometer. Since morphine-nembutal anesthesia has a tendency to lower body temperature, it was possible to study the temperature gradients at different rectal temperatures. The table below presents the mean temperatures and gradients obtained in 19 experiments on 11 animals. The rectal temperature was used as the point of reference in determining the gradients.

LOCATION OF CATHETER	$^{\circ}F$	A(X − R)
Rectum	99.1	
Rt. ventricle	98.7	−0.44
Pulmonary artery	98.7	−0.40
Coronary sinus	98.9	−0.20
Femoral artery	98.3	−0.84
Femoral vein	96.6	−2.51
Thigh muscle	97.6	−1.47
Thigh; subcutaneous	96.5	−2.64
Thigh; skin	92.1	−7.04

The work of the heart was altered variously with intravenous injections of epinephrine, aminophylline and methedrine and with inhalations of amyl nitrite. The temperature of the blood in the right heart during the action of these drugs was determined and the data will be discussed.

Determination of filtration rate and renal plasma flow in dogs without analysis of urine. C. Riley Houck. *Division of Physiology, Univ. of Tennessee, Memphis, Tenn.* The renal plasma clearances of sodium p-aminohippurate (PAH) and mannitol were determined simultaneously by *1*) the plasma slope method without urine analysis, and *2*) the conventional urine collection method, and the values compared. According to Newman,

Bordley, and Winternitz (*Bull. Johns Hopkins Hosp.* 75: 253, 1944), the renal clearance (C) of a substance is the product of its volume of distribution in the body (Vc) and the rate of disappearance from plasma (S). Under nembutal anesthesia, 4 dogs were injected intravenously with 5 gm. of mannitol and 750 mg. of PAH through a calibrated syringe. After 40 minutes, 5 10-minute blood samples were drawn, during which time urine was collected through an indwelling bladder catheter. In order to test for extra-renal loss of these substances, the dogs were subsequently bilaterally nephrectomized and 5 more 10-minute blood samples drawn. The urine clearance/slope clearance ratios for the 4 dogs were: for mannitol 0.940, 0.923, 0.956, and 0.781 (av. 0.90); and for PAH, (no determination on first dog), 1.052, 0.956, and 0.872 (av. 0.96), respectively. In every case, extra-renal disappearance of PAH and mannitol was zero. Except for one dog, it is apparent that the slope clearance of either PAH or mannitol can be substituted for the respective urine clearance. Consequently, it may be concluded tentatively that glomerular filtration rate and renal plasma flow can be determined in dogs by the analysis of plasma alone without the collection and analysis of urine.

Disappearance of mannitol and PAH from plasma of bilaterally nephrectomized dogs. C. RILEY HOUCK. *Division of Physiology, Univ. of Tennessee, Memphis, Tenn.* For mannitol and sodium p-aminohippurate (PAH), the volume of distribution (Vc), the rate of disappearances from plasma (slope or proportion of Vc cleared of each substance per minute), and the clearance (extra-renal) (C) were determined in 16 bilaterally nephrectomized dogs under nembutal anesthesia according to the procedure of Newman, Bordley, and Winternitz (*Bull. Johns Hopkins Hosp.* 75: 253, 1944). Immediately after nephrectomy, 65 mg. of PAH (16 dogs) and/or 1700 mg. of mannitol (8 dogs) were injected rapidly intravenously from a calibrated syringe. After 40 minutes, from 5 to 8 venous blood samples were drawn at 10-minute intervals and analyzed. For mannitol, Vc = 246 cc/kg., S = 0.0785%/min., C(= VcS) = 0.168 cc/min/kg. For PAH, Vc = 280 cc/kg., S = 0.289%/min., C = 0.796 cc/min/kg. It is apparent that the volumes of distribution of PAH and mannitol are very similar. Furthermore, the extra-renal loss of PAH from plasma is greater than the loss of mannitol. When compared to the total urine (renal + extra-renal) clearances in normal dogs (Houck, *Federation Proc.* 7: 339, 1948), the mannitol clearance in these nephrectomized dogs (extra-renal clearance) is only 4%, and the PAH extra-renal clearance only 6% of the respective total urine clearance. Consequently, in calculating the clearance of PAH and mannitol in intact normal animals, by use of the slope method, the extra-renal clearances can be ignored because the extra-renal loss is negligible.

Vascular perfusion of the prostate gland of the dog. PERRY B. HUDSON (by invitation), W. W. S. BUTLER III (by invitation), HERBERT BRENDLER (by invitation) and W. W. SCOTT. *James Buchanan Brady Urological Inst., Johns Hopkins Hospital, Baltimore, Md.* For the first time a reliable surgical technique has been devised which allows perfusion of the prostate gland through its normal arterial and venous channels. This preparation is accomplished by ligation of all branches of the abdominal aorta and inferior vena cava except for the arteries and veins on each side which constitute the vascular supply to the prostate gland. The autonomic nerve supply may be preserved. The urethra is preserved permitting cannulation for collection of prostatic fluid during perfusion of the gland. This preparation is proving useful in the study of the metabolism of the dog prostate gland, offering certain advantages over purely *in vitro* techniques.

Active salt transport and its regulation in the isolated frog skin. ERNST G. HUF (introduced by ERNST FISCHER). *Baruch Center of Physical Medicine and Dept. of Physiology, Medical College of Virginia, Richmond, Va.* Freshly isolated frog skin actively transports salt from its natural outside to its natural inside (accumulation effect). Experiments have been carried out using 'inside out' skin bags, formed of the skin of the hind limbs. The bags were filled with 5 ml. of Ringer solution and immersed in 2.5 l. of Ringer solution of the same concentration; this was varied in different experiments from .4 times up to 2.0 times its normal concentration. After 12 hours, less fluid of a lowered salt concentration was left in the bag. The weight of the filled bag at the end of the experiment was always lower than the weight at the beginning, indicating a transport of fluid through the skin. An increase in weight of the bag was observed in experiments where the Ringer solution was poisoned with NaCN (.001 M). In this case the amount and the concentration of the Ringer solution placed in the bag was not diminished appreciably at the end of the 12-hour period. The accumulation effect is greatest when the skin is exposed to diluted Ringer solution and decreases with increasing concentration of the Ringer solution. Apparently the concentration of the salt solution regulates the mechanism of accumulation of salt. The endocrine and the nervous system seem to have little regulatory influence.

Toxins of Streptococcus pyogenes and cell respiration. F. R. HUNTER (introduced by A. K. PARPART). *Dept. of Zoological Sciences, Univ. of Oklahoma, Norman, Okla.* The effect of toxins of *Streptococcus pyogenes scarlatenae* on the rate of

O_2 consumption of several types of cells was studied. Almost complete inhibition was found using chicken erythrocytes, dogfish (*Mustelus canis*) erythrocytes, *Asterias* eggs and fertilized *Arbacia* eggs, the rate of respiration in the last instance being essentially the same as that of unfertilized eggs. Since it has previously been suggested that unfertilized *Arbacia* eggs respire through a non-ferrous, autoxidizable carrier while fertilized eggs have an active cytochrome system, the present data would suggest that the scarlatenal toxin reacts in some way with the cytochrome system. Confirmation of this suggestion was obtained from experiments involving chicken erythrocytes and the respiratory carriers, pyocyanine and methylene blue. The rate of O_2 consumption of control chicken erythrocytes was increased slightly by the addition of pyocyanine or methylene blue while the rate of respiration of erythrocytes which had been inhibited by the toxin was accelerated to the same level as the controls by the addition of these carriers. These experiments demonstrate that the toxins of *Str. pyogenes* inhibit the respiration of several different types of cells by reacting with the cytochrome system.

Electrical and functional activity of spinal motor neurons. JOHN HUNTER, RICHARD LILLIE (by invitation), and ROBERT GESELL. *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* The ventral root of the phrenic nerve of the dog exhibits two characteristic types of electrical activity: *1*) slow periodic waves which increase and decrease in negativity with inspiration and expiration respectively; and *2*) quick potential changes superimposed upon the inspiratory fraction of the slow waves. The slow potentials diminish in magnitude with distance from the cord and are thus concluded to spread from the cord by electrotonus. The quick potentials suffer no decrement and are concluded to represent the inspiratory action potentials. Continuing unbroken electrotonic activity in the phrenic root indicates that the dendrites and cell body are always negative with respect to the neuraxon and that neurocellar currents consequently circulate between dendrites and neuraxon via internal and external circuits. Waxing and waning of this continuing current produces periodic changes of intensity of the slow electrotonic component in the phrenic root. Rise of negativity of the slow component is attended by increasing numbers of action potentials. Sporadically and artificially increased depth of inspiration are attended by increased magnitude of the electrotonic component and by correspondingly increased numbers of action potentials. It is postulated that the current circulating between the dendrites and neuraxon generates nerve impulses at or in the proximity of the axon hillock. The current is therefore designated 'generating current'. It is suggested that our concept of specialization of function of the neuron questions the need of retaining the theory of synaptic transmission of nerve impulses and subsequent conduction along dendrites and cell body.

Proprioception and convulsions. J. HYDE, J. GAY and E. GELLHORN. *Laby. of Neurophysiology, Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* The influence of proprioceptive impulses on cortically induced afterdischarge and on chemically induced convulsions has been studied on cats and monkeys (Macacus Rhesus) in dial anesthesia. Fixation of a joint so as to prevent shortening of a muscle during contraction leads to an increased afterdischarge as well as increased amplitude of EMG in response to a standard cortical stimulus. In contrast to this proprioceptive facilitation of afterdischarge, removal of proprioception by appropriate posterior root section decreases afterdischarge and in some cases qualitatively changes it from a tonic to a clonic form. Afferent proprioceptive impulses induced by stimulation of the peripheral end of the motor root S_1 have been seen to alter convulsive spikes induced by topical application of strychnine on the cortex. The effect is largely confined to the contralateral sensori-motor cortex and is greater in the hindleg than in the foreleg and face area. It consists in either increase or decrease in frequency of the strychnine potentials. Finally, convulsive movements induced by applying strychnine to the exposed dorsal columns of the cord are reduced or eliminated by deafferentation although not significantly altered by removal of cutaneous impulses. The experiments furnish evidence for the fact that convulsive potentials and movements are greatly influenced by proprioceptive impulses.

Determination of cardiac output by an improved electrical conductivity method. RAYMOND C. INGRAHAM. *Dept. of Physiology, Univ. of Illinois, College of Medicine, Chicago, Ill.* Basically the present method follows H. L. White's recent modification rather closely. A small quantity of hypertonic salt solution is injected as rapidly as possible into the right atrium. Conductivity changes are recorded in blood flowing through a specially designed conductivity cannula tied in a peripheral artery. The output of the bridge, of which the conductivity cannula constitutes one arm, is amplified, rectified and then recorded by the deflection of a suitable sensitive reflecting galvanometer. The degree of bridge imbalance is optically recorded and later translated to concentration changes of sodium chloride by the construction of a calibration curve. The improvements in the present method are essentially in the design of all constituent parts so that it accomplishes the desired purpose to a degree consistent with modern

electronic methods. Comparison of cardiac output as determined by this method in the intact animal with the direct Fick and dye injection method has yielded results that compare very favorably. Determinations were made simultaneously. It was found that the improved design required the use of a smaller amount of a more dilute solution of sodium chloride than previous applications of this method have required. This minimizes the error due to salt loss in passage through the lungs. Recirculation can be recognized, if it occurs, and corrections made. This method thus allows for continuous recording of cardiac output data and also simultaneous optical recordings of pressure variations in any accessible portion of the cardio-vascular system.

Dynamics of evaporative heat loss for a rapidly cooling human body. TOHRU INOUYE (by invitation), NATHANIEL GLICKMAN, and ROBERT W. KEETON. *Dept. of Medicine, Univ. of Illinois, Chicago, Ill.* The dynamics of evaporative heat loss for a rapidly cooling human body in a 'comfortable' environment after having been exposed to a hot environment was studied on 6 healthy young men. The subjects were clothed in 90% cotton union suits containing variable amounts of moisture accumulated as a result of 1 or 2-hour exposures to a hot environment. The hot room was maintained at 37°C. with a water vapor pressure of 41.1×10^3 dynes/cm.^{2} and the 'comfortable' room at 24.4°C. with water vapor pressures of 9.1×10^3, 18.2×10^3, and 24.3×10^3 dynes/cm.^{2} with air velocity minimal and constant. Observations included weight loss and skin temperatures obtained at short intervals for 1 hour. The equation governing evaporative heat loss was theoretically derived for the dynamic state. Hitherto, this was applied only on subjects in approximate equilibrium with the environment with the introduction of wettedness as a calculated factor to account for an indeterminate variable. Under dynamic conditions, available moisture in the union suit and on the skin was found to adequately express wettedness. The inclusion in the equation of the amount of available moisture yielded a linear correlation for each of the 36 tests with an average correlation coefficient of 0.992. Thus, for subjects under rapidly changing physiological conditions, the amount of evaporative heat loss was proportional to the gradient of water vapor pressure between the skin and the ambient environment, amount of available moisture on the skin and in the union suit, surface area and time.

Developmental relationships between electrical activity and histogenesis of the cerebellum. JOHN JACOBS and RAY S. SNIDER (introduced by H. W. MAGOUN). *Depts. of Pathology and Anatomy, Northwestern Univ. Medical School, Chicago, Ill.* Correlation studies have been made between the first appearance of the fast intrinsic spontaneous electrical activity in the cerebellum of the newborn and the histogenesis of the cerebellar cortex. Anesthetized or acutely decerebrated newborn guinea pigs and rats between 1 and 28 days, as well as rat fetuses, were used. A complete comparative neuro-electrogenetic study on reptiles, avians and mammals, including primates, is in progress. Electrical activity was visualized and photographed from a cathode ray tube after amplification through a Grass Model III amplifier. Both bipolar and monopolar silver wire and cotton wick electrodes were used. For histogenetic studies silver, Nissl, Golgi and Feulgen (for thymonucleic acid) stains were used on Purkinje, granule and basket cells. Typical cerebellar electrical activity of 10–60 microvolts with a frequency of 150–250/sec. first appears about the 12th day postpartum in the rat and is present in the newborn guinea pig. Several histogenetic changes take place in the cerebellum of the rat about the 12th day: *1*) connections of the granule cell glomerulus are established; *2*) nuclear chromatin of the Purkinje cell first appears Feulgen positive; *3*) Purkinje cell nucleolus shows a trace of thymonucleic acid; *4*) cytoplasm of the Purkinje cell is Feulgen negative but gives a positive Nissl reaction as early as 10 days. Granule cells are Feulgen positive as early as 9 days. Data are not complete on basket cells.

Antihemolytic action of a hemolytic agent. M. H. JACOBS, MARIAN WILLIS LEFEVRE (by invitation) and CAROLYN STOUT (by invitation). *Dept. of Physiology, Univ. of Pennsylvania, Philadelphia, Penna.* Sodium oleate shows a notoriously complex relation between concentration and hemolytic activity and according to circumstances increasing its concentration may either increase or decrease the rate of hemolysis. This and other complications are due, at least in part, to the coexistence of a hemolytic and an antihemolytic effect of the oleate. The hemolytic effect has the general characteristics of an induced cation permeability, as shown by shrinkage of the cells in isosmotic sucrose, protection by low concentrations of sucrose, rapid return of volume after shrinkage by concentrated NaCl and swelling in alkaline solutions. This effect, which increases with concentration, is more or less disguised by the antihemolytic effect, which also increases with concentration and seems to be due to the prevention by oleate at the cell surface either of swelling of the cells or escape of hemoglobin, or both. The antihemolytic effect is well shown in photographically recorded hemolysis curves. If during hemolysis by weak oleate, more oleate is suddenly added the process may instantly, though temporarily, be stopped. Osmotic hemolysis may similarly be

checked. Conversely, removal of protective oleate by appropriate dilution with isotonic NaCl causes immediate hemolysis, provided that the initial exposure has been sufficient to render the cells cation permeable. A more striking way of showing the same thing is by the addition of washed normal erythrocytes to a suspension of other erythrocytes that have been altered but simultaneously protected by oleate; under properly chosen conditions hemolysis of the treated cells then immediately occurs.

Influence of niacin on the excretion of ketone bodies in normal fasted rats. R. G. JANES and J. BRADY (by invitation). *Dept. of Anatomy, State Univ. of Iowa, Iowa City, Ia.* In previous experiments we have shown that relatively large amounts of niacin will produce a ketonuria in severely diabetic male rats. In the present study it has been found that the daily administration of 35 mg. of niacin to normal female rats was ineffective in altering acetone body excretion. However, if female rats were given daily intraperitoneal injections of niacin during a 5-day fasting period, a marked ketonuria occurred. Control fasted rats receiving injections of physiological saline failed to show a pathological level of ketone body excretion. Niacin has been given to rats at levels of 5, 20, 35 or 50 mg. per day. All of these animals showed greater acetone body excretion levels than control rats but the 35- and 50-mg. dosages appeared to produce a more marked ketogenic response although some variation was seen in each group.

Regulation of food intake in normal and esophagostomized dogs. HENRY D. JANOWITZ (by invitation) and M. I. GROSSMAN. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Factors concerned in regulating intake of food were investigated in dogs by noting *1*) effects of intragastric feeding on normal feeding in dogs with gastric fistulas, *2*) effects of real and sham feeding on eating time in dogs with esophagostomies and *3*) effects of oral supplementary prefeeding on food intake in intact animals.

Under standardized conditions, intragastric feeding of 50% or more of the average daily meal had an immediate significant but not equivalent depressing effect on oral food intake. If time was allowed for gastric evacuation (4 hours) the feeding had no such inhibitory effect. Division of the exteriorized esophagus in otherwise normal dogs led to immediate significant prolongation of eating time. Satiety conferred by sham feeding was of short duration, usually less than two hours. Beyond this point, increasing food deprivation or lengthening intervals between sham feeding did not increase the duration of sham feeding. Intragastric feeding and absorption of such feeding (up to 4 hours) had no inhibitory effect on sham feeding time. Sham feeding time was shortened but not reduced to normal by simultaneous sham and intragastric feeding. Gastric distention by ballon reduced sham feeding time only when obvious nausea was induced. The oral prefeeding of a fraction of the daily meal reduced food intake by a corresponding amount in intact animals.

These studies disclose two sets of factors which under these standardized conditons influence regulation of intake of food *1*) oral factors ('gustatory satiety') and *2*) gastric distention factors, which to be effective must act simultaneously with the oral factors.

Action of Phenylindanedione on prothrombin time. L. B. JAQUES, E. TAYLOR (by invitation) and E. LEPP (by invitation). *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada.* Meunier, Mentzer and Molho (*Compt. rend. Acad. d. sc.* 224: 1666, 1947) have reported that 2-phenyl indane dione-1,3 (PID) is a more rapidly acting prothrombopenic agent than dicumarol. The effect of PID on prothrombin time was tested in dogs and rabbits. Single doses showed a rapid but slight effect on prothrombin time. Repeated administration of the drug at frequent intervals caused a marked effect on the prothrombin time. 8.3 mg/kg. every 8 hours gave a prothrombin time of infinity, 1 mg/kg/8 hours doubled the prothrombin time. Withdrawal of PID caused the prothrombin time to return to normal within 36 hours. Vitamin K had no effect on the action of PID.

The prolonged prothrombin time following administration of PID was accompanied by a normal concentration of prothrombin in the blood but there occurred a very marked diminution in *Factor V* activity (Owren). The concentration of *Factor V* itself was not reduced, suggesting the presence of an inhibitor of *Factor V*. PID itself had little effect on the components of the clotting system. Large doses of the drug over a long period of time plus other factors produced a hemorrhagic condition similar to that of dicumarol overdosage. Withdrawal of the drug rapidly reversed this condition. No toxic effects were observed with 8.3 mg/kg. given every 8 hours for 48 days to dogs. The PID was excreted by the kidneys.

Studies on 'dumping syndrome' in the dog following anastomotic operations and vagotomy. N. C. JEFFERSON (by invitation), C. W. PHILLIPS (by invitation), R. LEVINE and H. NECHELES. *Dept. of Gastrointestinal Research, Medical Research Inst., Michael Reese Hospital, Chiago, Ill.* Following a study on patients suffering from the so-called dumping syndrome after subtotal gastrectomy (*Gastroenterol.* Feb. 1949), the present study on dogs was done in order to obtain more controlled conditions. Intravenous and oral glucose and fructose tolerance tests were performed before and

after operation, and in some tests emptying time was determined with the barium meal. The following anastomotic operations were done: Polya, Hofmeister, gastro-enterostomy, pyloro-plasty, entero-enterostomy and additional vagotomies. The following results were obtained: in no normal control dog did hypoglycemia develop with any of the tolerance tests. In none of the operated animals did the tolerance test evoke hypoglycemia. Subsequent to anastomotic operations the oral glucose tolerance curve showed a persistent increase in height and duration. In most animals in which bilateral transthoracic vagotomy was performed with or subsequent to anastomotic operations, the oral glucose tolerance curve showed an additional increase in blood sugar levels. Oral glucose tolerance tests on 2 dogs with subtotal gastrectomy held in the upright position during the test revealed increased height of the glucose tolerance curve. In 2 animals with anastomotic operations in whom following vagotomy an increased glucose tolerance curve occurred, atrophy of the pancreas was found at autopsy.

Further studies on electrical analogs of the circulatory system. KENNETH E. JOCHIM. *Dept. of Physiology, Univ. of Kansas, Lawrence, Kansas.* In a previous report simplified electrical analogs of the circulatory system were described, and results of an analytical and experimental study of their behavior were presented. These circuits consisted of single or multiple mesh networks which were energized with half sine wave current pulses, and in which the distensibility of the arterial system was represented by one or more parallel condensers.

This study was extended by introducing two additional complications which simulated more closely the conditions in the circulatory system: *1*) the networks were energized with current pulses of various wave-forms, and for each one it was shown, experimentally and analytically, that the ratio of pulse volume, or maximum arterial uptake, to stroke volume (using hemodynamic terminology) fell between 0.50 and 0.56 over a wide range of values of the circuit parameters. A graphical method was evolved for computing this ratio for any input wave-form, whether its equation is known or not. *2*) It has been shown by Hamilton and by the author that the distensibility curves of rubber tubes and arterial segments depend on the rate of change and direction of change of applied tension. This type of distensibility variation was simulated electrically and its effect on the pulse volume/stroke volume ratio was shown, analytically and experimentally, to be insignificant within certain limits.

Cause of injury in rapid deceleration. MILTON JOFFE (by invitation) and FRED A. HITCHCOCK. *Dept. of Physiology, Ohio State Univ., Columbus, Ohio.* Experiments have been carried out on cadavers and anesthetized dogs to determine the effects of rapid deceleration. Acceleration was accomplished by falling weights attached by a steel cable to the lower extremity of the test body. Rapid deceleration was produced by a safety harness attached around the waist and connected by means of a tail line to the cross member of a T-shaped channel iron frame. The G forces produced were recorded photographically from vacuum tube type accelerometers. The decelerative forces were less than those expected from calculation, which was probably due to the lack of rigidity in the tube attachments. It was observed that there was no damping effect of tissues on the transmission of forces from the belt to the heart.

Seven dogs were subjected to multiple decelerations. Serial electrocardiograms were made on all dogs over a period ranging up to 9 days or until death. Autopsies were performed at death. Results of serial electrocardiograms on dogs showed inversions or other abnormalities of the T-wave in one or more of the 3 standard leads. X-ray heart shadow in one case, autopsy in all 7 cases, showed a dilatation of the heart with thinning of right ventricular wall. Four of 7 dogs also showed pulmonary, hepatic or renal vascular engorgement. The findings pointed to a condition of right heart failure—the traumatic heart injury syndrome.

Influence of hydrostatic pressure and of urethan on the thermal inactivation rate of bacteriophage. FRANK H. JOHNSON and RUTH A. C. FOSTER (by invitation). *Biological Laby., Princeton Univ., Princeton, N. J.* The activity of *Escherichia coli* bacteriophages T-1, T-2, T-5 and T-7 is destroyed at 60 to 68°C. in nutrient broth, pH 6.6, at rates which change with time during the exposure to heat. Addition of 0.005 M $MgCl_2$ retards the rate of destruction of infective centers of T-1, T-2, T-5, but not of T-7, and results in a first order rate of destruction of T-5. Urethan in concentrations of 0.05 to 0.5 M accelerates the rate of thermal destruction of all 4 of these bacteriophages. Increased hydrostatic pressure, up to 10,000 lb. p.s.i., retards the rate of thermal inactivation of T-1, T-2, and T-5, but slightly accelerates that of T-7. In presence of urethan, pressure opposes the rate of destruction of T-5 but has little effect on that of T-7.

An intact mammalian muscle preparation for use in studies on respiratory metabolism. SHIRLEY A. JOHNSON (by invitation) and KENNETH C. FISHER, *Univ. of Toronto, Toronto, Canada.* An examination of the properties of the isolated rat diaphragm lead to the conclusion that this preparation is far from representing normal resting muscle. We have therefore given consideration to other, possibly more normal, preparations. It has been found that the

extensor digitorum longus from the lower leg of 50 to 70-gm rats is a very satisfactory preparation. At 30°C. it always remains excitable for at least 6–8 hours, and in pure oxygen the rate of diffusion of oxygen into the preparation is sufficient to maintain the normal rates of consumption. Though this muscle appears to be more satisfactory for use than is the diaphragm, even it is probably not strictly resting tissue.

Suppression of urinary chorionic gonadotrophin, symptoms and pulmonary metastases in chorionepithelioma by diethylstilbestrol. EDWIN C. JUNGCK (by invitation), WILLIAM O. MADDOCK (by invitation), CLIFFORD L. FEARL (by invitation), and CARL G. HELLER. *Depts. of Physiology, Gynecology and Medicine, Univ. of Oregon Med. School, Portland, Oregon.* Large doses of stilbestrol are effective in suppressing anterior pituitary gonadotrophin secretion in post-menopausal and castrated women. Therefore, stilbestrol was administered to a 28-year-old woman with pulmonary metastases from a uterine chorionepithelioma, in hope of similarly depressing the gonadotrophins of chorionic origin, and with the vague hope that suppression of function might be accompanied by morphological regression of the malignant cells. At the time the primary tumor was removed (total utero-salpingo-oophorectomy), no pulmonary metastases were visualized. Four months later, multiple pulmonary lesions characteristic of metastatic chorionepithelioma were noted along with weight loss, asthenia and hemoptysis. Six months after operation, urinary excretion of chorionic gonadotrophin was 3200 I.U./24 hours. After 4 weeks of daily intramuscular injections of 10 mg. of stilbestrol in oil, chorionic gonadotrophin excretion dropped to 640 I.U., symptoms disappeared and pulmonary metastases were regressing. In 9 weeks, hormone titers were 6–16 I.U./24 hours and remained at that level as long as daily injections were continued; radiological evidence of pulmonary metastases was no longer present. Stilbestrol, 10 mg. in water suspension, was as effective as the oil preparation when given intramuscularly. After 6½ months, 25 mg. stilbestrol daily by mouth was substituted for the injections. A slight rise in titer was observed, which disappeared when the dose was increased to 50 mg. daily by mouth.

The above findings could be due to coincidence; however, they suggest that *1*) stilbestrol may inhibit chorionic as well as pituitary gonadotrophins and *2*) morphological and functional regression may accompany each other when stilbestrol is administered in chorionepithelioma.

Site of action of myanesin (tolserol) in the central nervous system. B. R. KAADA (introduced by JOHN F. FULTON). *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Following the announcement of the muscular relaxation effect of Myanesin (3-ortho-tolosy-1,2-Propanediol) by Berger and Bradley in 1946 there has been marked interest in the potential value of this drug as an adjuvant to anesthesia and in the treatment of neuro-muscular disorders such as rigidity, spasticity, muscle spasm and involuntary movements. The site of action of this drug, particularly in clinical doses (20–30 mg/kg.), is as yet unknown. With such doses of Myanesin it has been confirmed that hyperirritable spinal reflexes (in our study experimentally produced in cats and monkeys under light chloralose anesthesia, by injection of prostigmine and strychnine) can be reduced, but are without any effect on normal reflexes. In the present study it has been shown that at higher dosage (40–50 mg/kg., i.v.) the multi-neuron reflex discharges are depressed before the two-neuron ones, and correspondingly flexion and crossed extension reflexes are depressed earlier than the knee jerk. In spinal animals strychnine increases predominantly the multineuron reflex discharge, which can be reduced to normal promptly with Myanesin. Complete inhibition of strychnine convulsions has also been obtained from electrical stimulation of the bulbo-reticular suppressor region and facilitation of subconvulsive doses from the facilitatory region. The multineuron discharges can be inhibited or facilitated at lower stimulus intensities than the two-neuron reflex discharge from these regions. The threshold for obtaining suppression or facilitation of spinal reflexes from stimulation of the bulbar suppressor and facilitatory centers was measured before and after the administration of clinical doses of Myanesin. The threshold was found decreased for the suppressor and increased for the facilitatory stimuli, indicating that Myanesin in some way affects the balance between inhibitory and facilitatory impulses playing upon the spinal neurons in favor of the former. The possibility that Myanesin at least partly may act via or on the same structures as the suppressor or facilitatory systems is suggested.

Respiratory and vascular responses in monkeys from temporal pole, insula, orbital surface and cingulate. B. R. KAADA, K. H. PRIBRAM and J. A. EPSTEIN (introduced by JOHN F. FULTON). *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* The results of electrical stimulation of the cortex of the temporal and frontal lobes and the insula of monkey with recording of vascular and respiratory responses are reported. Square wave pulses of low intensity (3–8 volts) and of varying frequency and pulse duration were used. From the temporal pole blood pressure alterations and respiratory inhibition were obtained.

The optimal parameters of stimulation were found to be a frequency of about 40/sec. and long pulse durations (10 msec.). With these optimal parameters similar vascular and respiratory responses were obtained from a continuous cortical region extending through the anterior insula, the posterior orbital surface of the frontal lobe, the subcallosal region to the rostral limbic gyrus and also including basal olfactory structures (uncus, limen insulae, anterior perforated space). The lateral surface of the frontal lobe including the depths of the arcuate and principal sulci were explored, but only inconstant respiratory changes were obtained from the cortex dorsal to the superior limb of the arcuate sulcus and ventral to its inferior limb. The continuous responsive region is covered either by allocortex or by agranular or sparsely granular isocortex showing some characteristics of the nearness of allocortex. The relevant connections of these areas are discussed.

Radioactive diiodotyrosine in studies of thyroidal and tissue organic iodine. GOPAL KARANDIKAR (introduced by WILLIAM T. SALTER). *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.* All mammalian tissues contain organic iodine (so-called 'thyrenzyme'), except when hypothyroidism follows thyroidectomy or prolonged treatment with a goitrogen. When radioactive *iodide* is administered, the thyroid gland forms radioactive diiodotyrosine; later the organic iodine in peripheral tissues increases while the radioactive iodide declines. After the administration of radioactive diiodotyrosine intraperitoneally to rats (both normal and goitrous), part of the ensuing changes in iodine metabolism is due to iodide originating from decomposed diiodotyrosine. This feature is dependent quantitatively upon the dosage employed, because in larger dosage relatively less diiodotyrosine is de-iodinated. Both thyroid gland and peripheral tissue share in the newly acquired iodine. In less than an hour after diiodotyrosine is injected the total iodine in peripheral tissues increases. Besides iodide, diiodotyrosine contributes to the increase; but in the early stages its association with protein fractions is largely by adsorption. Eventually some of the newly acquired iodine is incorporated in the tissue protein (e.g., muscle, liver), although the distribution is not uniform throughout the several muscle protein fractions. In the building up of the genuine ('thyrenzyme') peripheral protein-bound iodine the thyroid gland plays an important role, which is influenced by goitrogenic substances.

Bile action on the volume and content of gastric juice in triple histamine experiments. JERZY KAULBERSZ and RYSZARD BILSKI. *Dept. of Physiology, Jagellonian University, Cracow, Poland.* This study is a continuation of previous work done at Wayne Univ. College of Medicine by one of us (J. K.), which revealed in single histamine experiments (*Federation Proc.* 1: 45, 1942) that bile increases the secretion from the Pavlov pouches and gastric fistulas, while it seemed to have little if any effect on the secretion from the Heidenhain pouches. Beamer, Friedman, Thomas and Rehfuss (*Federation Proc.* 3: 3, 1944) noted that bile introduced into the stomachs of dogs with fistula results in a secretion of gastric juice, and instilled directly into the intestine it increases the gastric secretory response to proteoses.

In the present investigation a triple histamine method on dogs with Heidenhain pouches was employed, with one bile plus histamine and two histamine control tests on each day of the experiment. The total output of HCl and peptic power was determined. When histamine alone was administered 3 times a day in 24 experiments the average mEq. of the first and third periods were identical, those of the second period 5% lower. Bile solution (5–10% ox bile) was introduced through a gastric tube in 54 triple histamine experiments, 18 times in each period. It evoked an increase of the volume of secretion from the Heidenhain pouches, affecting only slightly the acidity and the peptic power. The average mEq. were 16–27% higher than those of the controls. Bile also exerts in dogs with Heidenhain pouches a slight stimulatory effect on the gastric secretion after histamine, which may not be easy to detect in single histamine experiments because of the daily variations in the HCl output. In cases of hypersecretion, the regurgitation of bile into the stomach may present a source of continuous stimulation.

Functional disturbances and their compensation following circumscribed lesions of the vestibular nuclei. S. R. KEMBERLING (by invitation) and E. A. SPIEGEL. *Depts. of Exptl. Neurology and of Pediatrics, Temple Univ. School of Medicine and Hospital, Philadelphia, Penna.* Circumscribed lesions of the left vestibular nuclei (V.N.) were produced in 46 cats; in 29 the symptomatology was studied up to 5 months. Definite vestibular symptoms developed in 5 cats without abnormal head posture. In 4 others torticollis remained the only symptom except for slight homolateral hypotonia demonstrable by measurements only. The localizing value of this dissociation remains to be analyzed histologically. Postrotatory nystagmus tested in 12 cats showed no disturbance in one, reduction to left in 5, transitory loss to left and replacement by a few jerks to right in 2 (appearance of latent spontaneous nystagmus to right?). Definite compensatory phenomena as observed following labyrinthectomy (Ruttin) were absent. In 4 cases, induced nystagmus to left was increased (effect of

latent tendency toward spontaneous nystagmus to left?). Residual slight reduction of extensor tone could be demonstrated only by measurements in 3 cats. Despite incomplete recovery of tone, decerebration could produce definite extensor rigidity of the forelimbs. Following decerebration there was in 3 cats no or only minimal difference of posture of the forelegs with slightly diminished extensor tone demonstrable by measurements only. In one cat measurements failed to reveal differences of left and rightsided extensor tone. In one instance maximum extensor tone (elbow) was higher on the operated side and twice extension was more pronounced in the left than the right hindleg (apparently overcompensation). The experiments point to the importance of the reticulate substance besides the V.N. for maintenance of tonus (Spiegel and Bernis, *Arbeiten Neurol. Inst.* Vienna, 1925).

Effect of oxygen administration on the survival of dogs explosively decompressed to 30 mm. Hg. JOHN P. KEMPH (by invitation) and F. A. HITCHCOCK. *Dept. of Physiology, Ohio State University, Columbus, Ohio.* It has previously been reported from this laboratory that dogs survive exposure to a barometric pressure of 30 mm. Hg for not more than 2 minutes. A series of experiments has been carried out in which oxygen was administered by insufflation to dogs explosively decompressed to this pressure. These dogs usually survived exposures of 3 minutes to this pressure. Insufflation was begun immediately after explosive decompression and continued until after the animal was returned to atmospheric pressure. Immediately upon recompression there was a bradycardia. The heart rate then gradually increased to a tachycardia, followed by a gradual decrease to a normal rate. Spontaneous respiration began about 60 seconds after insufflation was stopped. Occasionally animals survived 2 such explosions. In the animals which did not survive 3-minute exposures, several spasmodic ventricular beats were noted and autopsy occasionally showed the auricles to be contracting rhythmically. It is believed that this procedure is beneficial in two ways: *1*) the pO_2 of alveolar air is increased and *2*) the intrapulmonary pressure is maintained at a level sufficient to prevent the rapid evaporation of body fluids and to delay the formation of intravascular bubbles.

Influence of hesperidin methyl chalcone on the action of cardiac, striated and smooth muscles. W. JOHN KENFIELD and DAVID F. BOHR (introduced by ROBERT GESELL). *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* Observations have been made to determine the effects of hesperidin methyl chalcone (HMC) on the action of various isolated muscle tissues. One of the primary goals of this study has been to determine if the drug has any activity as a general protoplasmic toxin. The influence of HMC on the isolated sartorius muscle of the frog was studied by comparing the shape of its fatigue curves obtained in the presence of HMC with the shape of control fatigue curves. The presence of 0.2 mg. HMC per cc. in the Ringer's bath did not change the shape of the fatigue curves. A study was made of the amplitude of contraction of the turtle's ventricle perfused with Ringer's solution containing various concentrations of HMC. No decrease in amplitude of contraction was observed with concentrations varying from 0.02 mg. per cc. to 0.2 mg. per cc. The influence of HMC on the activity of smooth muscle was studied by observing its effects on isolated longitudinal muscle of the rabbit duodenum. In concentrations varying from 0.01 to 2.0 mg/cc. of Tyrode's solution, HMC caused a decrease in both muscle tone and amplitude of intrinsic rhythmic contractions. These changes were completely reversible. Although definite changes in the physiological activity of smooth muscle were observed, no evidence was obtained to indicate that HMC has a toxic effect on isolated tissues.

Factors influencing the demonstration of renal tubular potassium secretion in the normal dog. THOMAS J. KENNEDY, JR., JAMES G. HILTON, and ROBERT W. BERLINER (introduced by JAMES A. SHANNON). *Research Service, First (Columbia Univ.) Division, Goldwater Memorial Hospital, and the Dept. of Medicine, College of Physicians and Surgeons, Columbia University, New York City.* Evidence of renal tubular secretion of potassium has been previously presented (Berliner and Kennedy, *Proc. Soc. Exptl. Biol. & Med.* 67: 542, 1948; Mudge, Foulks and Gilman, *Ibid*, p. 545). Experiments involving infusion of K salts have been done on 10 normal dogs. Excreted potassium was compared with filtered potassium, estimated as the product of plasma K and glomerular filtration rate. The latter was measured as the clearance of creatinine, inulin, or thiosulfate which, measured simultaneously, give identical results under the circumstances of these studies. Direct evidence of secretion, manifested by an excess of excreted over filtered K, has been obtained in all 10 dogs. In only one of 8 dogs could this be demonstrated without previous oral administration of K salts. Even in K-tolerant dogs there are considerable individual differences in the magnitude of the excess of excreted over filtered. The ratio of excreted to filtered is a function of the anion with which K is infused—non-reabsorbable anions such as thiosulfate or ferrocyanide give considerably higher ratios than chloride. Within the range of plasma K safely attainable, there is no evidence of depression of the ratio of excreted to filtered as the rate of infusion of K is increased.

Sub-estrous response to dietary restriction and reversal in castrate C3H mice. JOSEPH T. KING, CARMEN B. CASAS (by invitation) and M. B. VISSCHER. *Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* The sub-estrous response seen in certain strains of ovariectomized mice when fed *ad libitum* can be inhibited by restricting the caloric intake to about 66% of normal (Casas, King and Visscher, *Federation Proc.*, 6: 87, 1947). Groups of 10 or more C3H mice, ovariectomized at 19–21 days of age, have been studied to determine the estrous response to full feeding after periods of restriction from 1 to 6 months; other groups were reversed from full feeding to restriction at periods up to 4 months. Control groups always full fed or always restricted were also studied. Restriction was to about 66% of the calories eaten by controls fed *ad libitum*; the absolute amounts of protein, salts and vitamins were essentially the same as taken by the control group. Smears were taken by the lavage method. In general restricted mice developed dense, mixed smears in 4–12 weeks after reversal to *ad libitum* feeding; there is considerable individual variation in the time required to develop a positive smear. Mice placed on restriction after full feeding show a positive smear for 2–5 months. If the period of full feeding is not longer than 1 month the mice usually remain in anestrous. Related weight changes are discussed.

Mixing rate within the mobile phosphate pool of cows. MAX KLEIBER, A. H. SMITH (by invitation), N. P. RALSTON (by invitation) and D. E. JASPER (by invitation). *College of Agriculture, Univ. of California, Davis, Calif.* Intravenous injection of P^{32} into cows allowed the measurement of turnover rates for various processes of phosphate transfer. These rates characterize the mobility of the phosphate pool. The turnover time for mixing the mobile phosphate in blood with that of the extra cellular tissue fluid of the cow is of the order of 10 minutes.

Procedure for the *in vitro* study of hormonal influences in nitrogen metabolism. DANIEL L. KLINE (introduced by JANE A. RUSSELL). *Dept. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven, Conn.* Rat diaphragm and liver slices were incubated in 4 cc. of Krebs-Ringer-bicarbonate solution at 38°C. and the nitrogen which appeared in the medium was determined at 15 and 195 minutes. To obtain results which were consistent and biologically meaningful, it was necessary to centrifuge from the medium fragments which separated from the tissues during incubation. The N which appeared in the medium during the first 15 minutes was non-metabolic in origin, arising chiefly as debris from slicing. This was shown by the failure of ionic, osmotic and temperature changes to affect the 15-minute value, whereas cutting the tissue into more pieces of the same thickness increased this value. The output of N during a period of 3 hours after an initial 15-minute equilibration period is believed to be metabolic in origin since the rate of addition of N to the medium during this time was lessened by lowering the temperature of incubation and was increased by the exclusion of O_2 and the addition of iodoacetate to the medium.

For normal animals the addition of total N to the medium between 15 minutes and 195 minutes was 0.31 ± .01 mg/100 mg. of wet diaphragm and 0.35 ± .02 mg/100 mg. of wet liver slices. The amino N increment in the medium was 52 ± 3 mcgm/100 mg. of diaphragm and 44 ± 2 mcgm/100 mg. of liver. In hypophysectomized rats the increments of total N were 0.18 ± .02 mg/100 mg. of diaphragm and 0.24 ± .03 mg/100 mg. of liver. The amino N increments were 29 ± 2 mcgm/100 mg. of diaphragm and 28 ± 2 mcgm/100 mg. of liver. These values are significantly different from those obtained in the normal series.

Isolation of legcoproporphyrin. HENRICH KLÜVER. *Division of the Biological Sciences, Univ. of Chicago, Chicago, Ill.* In a study of the prophyrins in the root nodules of legumes we have found that the nodules of *Phaseolus vulgaris* (var. Red Kidney bean) contain a porphyrin which appears to be characteristically different from previously described naturally occurring porphyrins. The absorption and fluorescence spectra of the free porphyrin and of its methyl ester as well as of various metal complexes are practically identical with those of coproporphyrin. As regards solubility, the legcoproporphyrin behaves like the Waldenström type of uroporphyrin. It can be transferred from aqueous extracts to ethyl acetate at a *p*H of 3.2 to 3.5. The legcoproporphyrin obtained from the nodules of plants 4–5 weeks old is chromatographically separable into two porphyrins on a $CaCO_3$ column. Different extraction procedures and rechromatography do not affect this separation into two porphyrins. It has not been possible to distinguish the two components spectroscopically. Our attempts at crystallizing the legcoproporphyrin ester or its components have failed although we have succeeded in crystallizing the esters of protoporphyrin and mesoporphyrin derived from the heme compounds in the same nodules. Microscopic and microspectroscopic examinations of sections through root nodules under ultraviolet clearly indicate that the legcoproporphyrin is confined to the cytoplasm of the infected cells. Future studies of symbiotic nitrogen fixation must consider not only the presence of leghemoglobin in the infected cells, but also the presence of such substances as legcoproporphyrin. It is of further interest that the organs and body fluids of

porphyria patients may contain a porphyrin which seems to possess the characteristics of legcoproporphyrin.

Oximetric method for determination of the oxygen saturation of mixed venous blood in man. JULIAN KNUTSON (by invitation), BOWEN E. TAYLOR (by invitation), and EARL H. WOOD. *Section on Physiology, Mayo Foundation, Univ. of Minnesota, Rochester, Minn.* Arterial oxygen saturation was recorded continuously and simultaneously in 6 subjects by both the modified earpiece oximeter and a whole-blood oximeter attached to an indwelling radial artery needle. The instrumental time lag was 2.5 and 2 seconds for the earpiece and whole-blood oximeter, respectively. Both oximeters were calibrated against Van Slyke analyses of arterial samples obtained when each subject breathed air, 14% oxygen and 100% oxygen. The Matthes procedure as modified by Elam and co-workers was used. This consists of deep expiration followed first by a deep inspiration of helium and, second, expiration to mid-position followed by about 30 seconds of breath-holding.

After the inspiration of helium, the initial decrease in arterial saturation (circulation time) occurred at 4.5 (3–7) seconds at the ear and at 4.6 (4–6) seconds at the wrist. Saturation decreased 17 (13–23) % in 10 (6–15) seconds and then reached a plateau in 3 of the subjects, or decreased gradually in the remainder until 4.8 (3–7) seconds (ear) and 7.5 (4–10) seconds (wrist) after the inspiration of air. The average duration and saturation at the arterial plateau was 4 (3–5) seconds and 78 (77–79) % at the ear and 4 (3–6) seconds and 75 (75–79) % at the wrist. In the 3 subjects in whom an arterial plateau was obtained, the cardiac index calculated by the Fick principle (assuming the plateau value equalled the saturation of mixed venous blood) was 3.1 (2.6–3.5) L/M²/min. The contour of the changes in arterial oxygen saturation recorded photoelectrically at the ear and directly on the arterial blood at the wrist were similar in every instance, indicating that the ear oximeter can be used successfully for determination of the arterial saturation plateau by the Matthes procedure.

Function of the ear in health and disease (motion picture). H. G. KOBRAK, JOSEPH E. HIND (by invitation) and ROBERT B. MILLER (by invitation). *Univ. of Chicago, Chicago, Ill.* The purpose of the film is to demonstrate the response of the ear to sound in health and disease. A method was developed at The University of Chicago by which it was possible to photograph the acoustic vibrations of the ear during the process of hearing. The method consists in proper optical magnification combined with stroboscopic illumination. The first part demonstrates the normal ear. Ear drum vibrations in response to sound stimulation are shown. Movements of ossicular chain and the vibrations of the round window membrane are demonstrated. The phase difference between the movements of the stapes in the oval window and the round window membrane is visualized. Diseases of the middle ear are demonstrated on fresh temporal bones. Pathological function due to inflammatory adhesions and due to stapes fixation are demonstrated. Hearing curves of certain types of deafness are shown and the response of the ear demonstrated. By using electronic filters, the sound perception of a diseased ear is given in the sound track similar to the actual hearing defect. Therefore, the audience is able to see the pathologic function on the screen and simultaneously hear the distorted acoustic signal which the patient perceives. The action of hearing aids and intensity changes due to the action of the hearing aid is demonstrated. The acoustical vibrations of a surgical 'fenestra' in the lateral semicircular canal are demonstrated. In the final scene, the response of the ossicular chain to a piece of orchestral music is given.

Some factors influencing the contractile process. SAUL R. KOREY (introduced by DAVID NACHMANSOHN). *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.* Investigations have been initiated to test factors which influence the contractile mechanism of the muscle fiber in contrast to those acting on the conductive mechanism. A most suitable material for studies on the contractile system are the muscle fibers prepared according to the method of Szent-Gyorgyi. In these fibers, kept in glycerol, conduction is abolished whereas contraction can be produced by ATP but not by electrical stimulation. Among a great number of chemical compounds tested, none were found to be able to produce contraction. This specificity of ATP supports Szent-Gyorgyi's assumption that the effect observed is basically identical with the physiological mechanism of contraction. Among the compounds which had no effect were adrenalin, acetylcholine and eserine. The activity of several enzymes in these fibers was studied, especially that of ATPase. Their relation to the contractile process will be discussed. The effect of some enzyme inhibitors was also investigated. Inhibitors of sulfhydral groups abolish the effect of ATP. The action of these inhibitors was found to be reversible. The possible interpretation of this observation will be discussed.

Skin resistance patterns associated with visceral disease. IRVIN M. KORR. *Kirksville College of Osteopathy and Surgery, Kirksville, Mo.* This abstract reports the results of a preliminary investigation of the part that visceral disease and irritations may play in determining the electrical

skin resistance patterns previously described (*Federation Proc.* 7: 67, 1948, and this issue). Two classes of patients having visceral disease have been explored with the dermohmmeter (Jasper) and found to have low-resistance areas (LRA) which not only showed segmental relation to the viscus involved, but were fairly consistent for a given disease entity and were related to the referred pain pattern. Patients who had had myocardial infarcts had LRA over 2 or more of the upper 4 thoracic vertebrae, near the sternum at corresponding rib levels, and over the medial edges of one or both scapulae in the corresponding dermatomes. In one subject, repeatedly examined over a period of months, such areas were first observed 3 weeks prior to a coronary occlusion. Patients with duodenal cap ulcer had areas of markedly lowered resistance over the spinal muscles, on the right side, opposite vertebrae T-5 to T-8, over corresponding ribs on the anterior chest wall and to the right of the umbilicus. In both diseases LRA appeared to coincide with or overlie the most painful or tender parts of the reference zone.

Experimental alterations in segmental sympathetic (sweat gland) activity through myofascial and postural disturbances. IRVIN M. KORR. *Kirksville College of Osteopathy and Surgery, Kirksville, Mo.* It was previously shown (*Federation Proc.* 7: 67, 1948) that patterns of electrical skin resistance over the trunk varied from individual to individual, but that areas of reduced resistance (hyperactive sweat glands), especially in or near the midline, remained relatively constant in each individual for weeks or months; the low-resistance areas (LRA) often showed a dermatomal distribution; were correlated in some subjects with reduced segmental motor reflex thresholds; overlay or adjoined areas of deep hyperesthesia; frequently showed 'trigger zone' characteristics. Subsequent investigations have been directed at the factors related to or contributing to segmental sweat gland hyperactivity. Dermohmmeter exploration of many subjects has shown the following: *1*) LRA occurred in segments in which there were known myofascial tenderness or rigidity, abnormal intervertebral relations, vertebral pathology, or other somatic or visceral irritations in the corresponding spinal segments. *2*) Clinical improvement was accompanied by elevation of the skin resistance and shrinking of LRA. *3*) Injection of 0.3 ml. 6% NaCl into erector spinae or intercostal muscle caused appearance of LRA throughout the corresponding dermatomes (spine to sternum) which outlasted the referred pain by many hours. *4*) Experimental postural imbalance induced by wearing a 'lift' under one heel for several hours produced new LRA in the segments in which pain and tenderness appeared, enlarged pre-existing LRA in other, often distant, segments and further reduced their resistance. These observations support the hypothesis that myofascial and postural disturbances are accompanied by and may induce or maintain segmental hyperactivity in the sympathetic nervous system.

Further studies on the effect of 'neurotripsy' on the partially denervated muscle of the dog. A. J. KOSMAN and J. N. FREDERICK (introduced by J. S. GRAY). *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, Ill.* In a previous communication (*Proc. Soc. Exptl. Biol. & Med.*, in press) we had reported that 'neurotripsy' (traumatic massage of a muscle) was ineffective in arresting the loss of strength and mass of the partially denervated anterior tibial muscle of the dog. The present investigation was undertaken in order to determine the effect of the 'neurotripsy' procedure upon muscles subjected to a more extensive denervation and observed for a longer period of time. Partial denervation of the anterior tibial muscles of 8 dogs was accomplished by the removal of the motor roots of L_5 and L_6 bilaterally. Closed manual 'neurotripsy' was performed on the anterior tibial muscle of one side 3 months following the initial lesion and the animals were then sacrificed 4 months following the 'neurotripsy'. At the time of death the mean tension and weight of the treated muscles were 2500 gm. and 7.29 gm., respectively, and of the untreated muscles, 2250 gm. and 6.77 gm. respectively. These differences are not statistically significant.

The spleen as a blood reservoir during hypoxia. KURT KRAMER and ULRICH C. LUFT (introduced by HARRY F. ADLER). *Dept. of Physiology, USAF School of Aviation Medicine, Randolph Field, Texas.* The response of the spleen to different types of hypoxia was studied in 20 dogs under light nembutal anesthesia. Continuous recordings were taken photoelectrically of arterial blood O_2 saturation and hemoglobin content. The spleen was exposed without interfering with vascular and nervous attachments and its weight recorded with an electric balance. Blood pressure tracings were taken by strain gage manometer. In acute hypoxia induced by breathing pure nitrogen the spleen did not lose weight until the arterial O_2 saturation was approaching zero. After that the organ contracted visibly and lost considerable weight. Simultaneously the systemic hemoglobin content increased. During recovery breathing air the spleen regained its initial size in about 20 minutes. In less abrupt hypoxia using the rebreathing method two phases of splenic activity were observed. Slow contraction commenced when the arterial O_2 saturation dropped below 40%. Shortly before failure of respiration and after the asphyxial rise in blood pressure

the spleen discharged a larger amount of blood as evidenced by a drop in weight of approximately 150 gm. and a rise in circulating hemoglobin of 3 gm. %. The inverse relationship between spleen weight and systemic hemoglobin was also observed during subsequent recovery when the excess hemoglobin disappeared while the spleen was refilling. In blood samples taken from the splenic vein during contraction hemoglobin concentration was 30–40 % higher than in arterial blood. Control animals in which the splenic vessels were ligated before the hypoxic episode showed no change of hemoglobin concentration in the general circulation.

Dark adaptation in lesions of the optic pathways. HOWARD P. KRIEGER (by invitation) and MORRIS B. BENDER. *Psychophysiological Laby., Dept. of Neurology, New York Univ. College of Medicine, New York City.* A study of the visual threshold for a luminous target, presented in a dark room, was made in patients with lesions of the optic pathways. These patients did not have disease of the retina. Preliminary perimetric studies, made under 7-foot candle illumination, revealed consistent defects in the fields of vision. Three patients had homonymous hemianapsias, a fourth an almost complete double hemianopsia and a fifth a temporal hemianopsia of the left eye. The machine used for testing was Wald's modification of the Hecht-Schlaer adaptometer. It was so altered that the source of light which acted as the target could be placed at any angle along any meridian in the subject's field of vision. All measurements were made on one eye at a time and in a dark room. Examination under these conditions disclosed that the luminous target was perceived by the patient in the apparently blind half-field. However, the final threshold in this region was about 500 times higher than in the corresponding normal region. Other significant findings were a great fluctuation in the threshold of the ability to see the target and a marked lag in the rate of dark adaptation. The patient with bilateral homonymous field defects showed similar changes in both fields of vision. The patient with the unilateral temporal field defect showed a change in the dark adaptation of the seemingly preserved temporal half. Parallel changes were found in other visual and sensory functions. In summary it was found *1*) that patients with lesions of the optic pathways were able to see a luminous target, presented in the dark, in their fields of vision which were apparently blind upon perimetric examination and *2*) that fields, apparently normal upon perimetric examination, may have changes in their dark adaptation process.

Control of early hypertension with dihydrogenated ergot derivatives and the physiological significance. W. G. KUBICEK, F. J. KOTTKE AND MILDRED E. OLSON (by invitation). *Dept. of Physical Medicine, Univ. of Minnesota, Minneapolis, Minn.* A significant reduction in arterial blood pressure was produced in young hypertensive patients following administration of dihydroergocornine methanesulfonate (DHO 180). A decrease of 10 to 20 mm. Hg in diastolic pressure persisted for the duration of the drug therapy (2–3 weeks) and gradually increased to the control level after removal of the drug. Since DHO 180 is known to have sympathicolytic and adrenolytic actions the above observations indicate that sympathetic nervous system activity may contribute to the establishment of early hypertension. Renal clearance tests revealed normal kidney function in these patients.

Adsorption and fluorescence characteristics of crustacean sinus gland extracts. ELOISE KUNTZ (introduced by IRWIN W. SIZER). *Arnold Biological Laby., Brown Univ., Providence, R. I.* The sinus glands of crustacea secrete one or more hormones which affect chromatophore activity, molting, oxygen consumption, phosphate metabolism, etc. Abramowitz (*J. Biol. Chem.* 132: 501, 1940) obtained a partially purified preparation which had the properties of a basic amine. This paper includes two methods of separation based on cation adsorption column techniques. An active principle is adsorbed by the sodium form of Amberlite IRC-50 at *p*H 5. It can be eluted with 0.1 N HCl. Another effective adsorbent is Folin-permutit which has been prewashed with 0.1% acetic acid. Elution is accomplished with 10% KCl. The strong fluorescence exhibited by the hormone in the oxidized form (maximum fluorescence with exciting light of 3450 Å) has been used to estimate the relative concentration of minute quantities of the purified sinus gland extract and can also be used to follow purification procedures. There is a linear relationship between concentration and intensity of fluorescence. The hormone is easily oxidized and reduced by mild redox systems. Glutathione oxidizes the reduced form. This oxidation is readily reversed by addition of sodium hydrosulfite to the hormone-glutathione mixture. Sodium hydrosulfite alone is ineffective in acid or neutral solutions but can reduce the oxidized form of the hormone in alkaline medium.

Mass spectrographic studies on expired and alveolar air. GEORGE H. KYDD III (by invitation) and FRED A. HITCHCOCK. *Dept. of Physiology, Ohio State University, Columbus, Ohio.* Studies made with the mass spectrograph developed in this laboratory for the continuous recording of the partial pressures of O_2 and CO_2 and already reported have shown that the pO_2, the pCO_2 and the R.Q. of alveolar air are all a function of the duration of exhalation. This work has been continued

and extended. In recent experiments maximal exhalations made in three seconds have been compared with similar exhalations lasting for 10 seconds or longer. In the longer exhalations the final pO_2 is always lower and the pCO_2 higher than in the shorter exhalations. The difference is often 10 mm. or more. In the longer exhalations the curves of both the O_2 and the CO_2 approach a plateau although they do not completely level off. Calculations of the R.Q. at one-second intervals confirm the previous report of Hitchcock and Stacy that the quotient during the first second is high and tends to decrease as the exhalation progresses. The value of the R.Q. toward the end of a maximal exhalation but not necessarily at the extreme end shows the best agreement with the R.Q. of exhaled air.

Transition of fibrinogen to fibrin. KOLOMAN LAKI (introduced by WILLIAM J. BOWEN). *Inst. of Biochemistry, Budapest. Laby. of Physical Biology, Exptl. Biology and Medicine Inst. National Insts. of Health, Bethesda, Md.* The transition of fibrinogen to fibrin, brought about by the action of thrombin, proceeds in 2 stages. Thrombin acting enzymatically is involved only in the first step, and its role is to modify the fibrinogen molecules. In the second step, the altered fibrinogen molecules polymerize to the fibrin clot. Iodination of fibrinogen results in preparations that do not polymerize any more but still respond to the action of thrombin with a shift in the isoelectric point. This shift very likely means that some acidic groups of the fibrinogen molecule are split off by thrombin. A difference between the acid-base titration curve of fibrinogen and that of fibrin shows that some groups of the fibrinogen molecules with a pK of about 9 are involved in the polymerization process. These could be either $-NH_2$ groups or the phenolic group of tyrosine. The polymerization is not simply an end-to-end association of the altered fibrinogen molecules. First these molecules polymerize to a unit with a particle weight of about 1.5 million and then these units, probably connected by electrostatic forces, build up the primary fibrils. In this respect, the polymerization of fibrinogen seems to be strikingly similar to the polymerization of actin, a protein of muscle fibril.

Attempt at direct measurement of values required for calculating the pulmonary diffusion coefficient for oxygen. CHRISTIAN J. LAMBERTSEN, JOHN K. CLARK (by invitation), DOMINGO M. AVIADO, JR. (by invitation), ROBERT G. PONTIUS (by invitation), EARL S. BARKER (by invitation) and JOHN MOYER (by invitation). *Laby. of Pharmacology, Univ. of Pennsylvania, Philadelphia, Penna.* Simultaneous, direct measurements were made of alveolar, arterial blood and mixed venous blood gas tensions and rate of O_2 consumption in a group of normal human subjects in an attempt to obtain data required for calculating of the pulmonary diffusion coefficient for oxygen. Subjects were studied while breathing air, 8% and 100% oxygen. The data obtained illustrated the important practical and theoretical difficulties to be expected in attempting direct measurement of the pulmonary diffusion coefficient for oxygen. The data provides values for alveolar and arterial O_2 tensions during the breathing of air, 8% and 100% oxygen, and a comparison of the several methods of alveolar gas tension determination employed. The removal of nitrogen from the lungs by 100% oxygen inhalation suggested the feasibility of using the end-expiratory Haldane-Priestly sampling method to obtain a close approximation to mean alveolar pO_2 in subjects breathing pure oxygen since, under these conditions, all alveoli should contain nearly equal and constant O_2 tensions. The difference in pO_2 between alveolar and arterial samples in 5 subjects breathing 100% oxygen ranged from 4 to 15 mm. Hg, indicating that a maximum of about 1% of the cardiac output has not been exposed to oxygen in the alveoli. The use of air, low oxygen and high oxygen inhalation may permit estimation of the relative importance of poorly ventilating alveoli and addition to pulmonary venous blood of blood which does not pass through alveolar capillaries.

The law relating blood flow to perfusion pressure. HAROLD LAMPORT. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Blood flow (I) in the hindlimb of a dog varies with the nth power of perfusion pressure (P). When vascular tone rises, n increases and the constant (C) in the formula, $I = CP^n$, shifts in a manner which needs to be correlated with the corresponding changes in vessel dimensions. This logarithmic pressure-flow curve characterizes pseudoplastic fluids. When slippage in the fluid does not occur (a discontinuous change in its flow character), the law of flow in a capillary of length L and radius R for pseudoplastic fluids is: $I = \frac{\pi R^3 m}{n+3}\left(\frac{RP}{2L}\right)^n$, where m and n are constants for the fluid in the tube. Perhaps blood, at high flow rates, should be considered to 'slip', the central streaming red cells with their longer dimension oriented axially being distinct from the surrounding plasma sheath. However, a gradual change in the flow character of the blood from vessel wall to axis seems more likely. Since the pressure-flow relation of blood is fairly well described by the same logarithmic expression which is effective for pseudoplastic fluids without slippage (oil-water emulsions analogous to blood are an example), the development for the glass capillary has been applied to the blood vascular system.

After allowing for the effects of branching of vessels, etc., an equation results which tested satisfactorily in a set of four pressure-flow curves on the same preparation under different degrees of vasoconstriction.

Tetraethylammonium and gastric motility of the dog. ARDELLE LANE (by invitation), C. R. ROBERTSON (by invitation), and M. I. GROSSMAN. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Gastric motility in the human is inhibited by tetraethylammonium (TEA). As the gastric secretory response to histamine is decreased by TEA in the human and not in the dog, this study was undertaken to determine if this species difference exists in regard to the effect of TEA on gastric motility. A series of x-ray studies on dogs given a barium meal was made with and without TEA (10 mg/kg. I.V.). Films were taken immediately after the meal and in the case of TEA administration immediately after the drug was given and at half hour intervals for a period of 2 hours and at hourly intervals until the stomach was empty. A series of x-ray studies on dogs directly after a barium meal and simultaneous administration of atropine and epinephrine (1 mg. of each) were taken as a control procedure for comparison with the effects of TEA. Gastric motility tracings before and after intravenous administration of TEA were made in gastric fistula dogs by the balloon water manometer method. In the x-ray studies gastric tone and emptying time were not appreciably changed by the administration of TEA. In the experiments in which atropine and epinephrine were given gastric tone fell as shown by x-ray. The kymographic records of gastric motility showed individual variation through motility was either unaltered or slightly inhibited for a few minutes. These studies indicate that in the dog gastric motility does not respond as in the human to the administration of TEA.

Electrocardiographic findings in anthracosilicosis. LEONARD P. LANG (by invitation) and HURLEY L. MOTLEY. *Dept. of Medicine, Jefferson Medical College and Cardio-Respiratory Laby., Barton Memorial Division of Jefferson Hospital, Philadelphia, Penna.* The incidence of right heart strain as manifest by electrocardiographic changes was correlated with physiological studies of pulmonary function, chest roentgenograms and fluoroscopic examination in over 150 patients with respiratory complaints and a history of prolonged exposure to dust inside coal mines (20–45 years). Postmortem examinations of miners have revealed that there is a high incidence of right ventricular hypertrophy and that many eventually die in cardiac failure. The physiological studies used for comparison with the electrocardiographic findings included: maximal breathing capacity, total lung volume, the degree of emphysema as measured by expressing the volume of residual air as percentage of total lung volume, and the mean partial pressure of O_2 in arterial blood (Riley method). Cardiac fluoroscopy was of little value in the study of these miners because the dense fibrous tissue masses in the hilar areas obscured the cardiac borders. Fixed rotation of the heart and fibrous adhesions complicated the interpretation of an electrocardiographic diagnosis of right ventricle hypertrophy. No apparent correlation was noted between the x-ray stage of silicosis as classified from the roentgenogram and the electrocardiographic changes. However, a relationship was observed between the incidence of right heart strain and the degree of emphysema based on quantitative measurements of residual air. In general the lower the resting mean arterial pO_2, the higher the incidence of right heart strain, however, short induced periods of acute hypoxia produced minimal electrocardiographic changes.

The effect of handometer exercise upon normal grip strength. ELEANOR M. LARSEN (introduced by W. J. MEEK). *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison, Wis.* The usual grip strength of normal individuals is determined largely by their occupation. If normal grip strength may be increased by Handometer exercise, the hand strength of individuals weakened by injury or disease might be expected to improve by employing the same method. The purpose of this investigation was to determine the effect of Handometer exercise upon normal grip strength. The usual hand strength of 14 young adult women, right-handed with one exception, was measured by a grip dynamometer before and after 20 bouts of work performed bi-weekly. The exercise apparatus was the Handometer which consists essentially of a sphygmomanometer-type hand bulb attached to an adjustable air-pressure system gauge. The work of compressing the bulb against a constant resistance of 30 lb. pressure was continued at the rate of 60 contractions per minute until the needle could no longer be held constant. The number of contractions were counted and recorded for each hand. The results for every subject consist of 3 grip dynamometer readings for each hand pre- and post-exercise, and 20 sets of Handometer data for each hand. The grip dynamometer data were analysed statistically. The results indicate that the usual right hand grip strength is stronger than the left; and that the Handometer exercise increased the grip strength in both right and left hands, with a slightly greater increase in the strength of the left hand.

Renal mechanism of albumin excretion in patients with nephrotic syndrome. HENRY D. LAUSON, FRANCIS P. CHINARD (by invitation), and

HOWARD A. EDER (by invitation). *Hospital of The Rockefeller Inst. for Medical Research, New York City.* Intravenous administration of Red Cross human plasma albumin to nephrotic children 3 to 5 years old has made possible a study of the mechanism of albumin excretion. The amounts given varied from 18 to 37 gm. Albumin concentrations in plasma and urine were estimated by Chow's immunological method (*J. Biol. Chem.* 167: 757, 1947). The plasma concentrations varied from 0.2 to 0.9 gm. per 100 ml. before infusion, increased by 1 to 2 gm. after infusion, and then fell during the next 24 to 48 hours, during which repeated clearances of albumin, and in some cases, inulin and endogenous creatinine were measured, together with plasma volume. The plasma volume varied in the same direction as the albumin concentration. In about half the experiments albumin clearance was approximately constant with all concentrations of plasma albumin. In the other experiments, however, during the first few hours after the albumin infusion, when plasma volume and albumin concentration were near the maximum, the albumin clearance rose 50 to 100%. Creatinine clearances did not show proportional increases, suggesting that the rise in albumin clearance was due to some cause other than those which control the glomerular filtration of water and crystalloids. The data are consistent with the interpretation that the cause of nephrotic albuminuria is increased glomerular permeability to albumin, almost all of the filtered albumin being excreted. The rise of albumin clearance is correlated with plasma volume expansion, and it may be that this expansion mediates a further increase in glomerular permeability.

The elasticity of the rat lung. RICHARD W. LAWTON AND ALLEN L. KING (introduced by JAMES D. HARDY). *Dept. of Physiology, Cornell Univ. Medical College, New York City, and Dept. of Physics, Dartmouth College, Hanover, N. H.* Changes are observed in elasticity of rat lungs with age. Retardation of growth by restriction of diet also alters the observed elastic characteristics of the lungs (Saxton, Barnes and Sperling, *J. Gerontology* 1: 165, 1946). Chronic pneumonia and bronchiectasis are eventually found in a high percentage of rats of advanced age. A mathematical analysis of elastic properties of rat lungs was undertaken, utilizing the data of the above authors, in order to evaluate the relation of the elastic changes to ageing and the disease process. A single alveolus is generalized as a hollow shell whose wall is composed of a mesh of rubber-like molecules arranged as irregular closed rings. The whole lung is considered as a multiple of the single alveolus. The equation is based on the current statistical mechanical theories of high polymers. $p - p_0 = A(r_0/r)^2 [L^{-1}\beta(r/r_0) - (r_0/r)^3 L^{-1}\{\beta(r_0/r)^2\}]$. In the equation p_0 and r_0 are the initial and p and r the distended pressure and radius respectively. The constant A is proportional to the number of elastic chains participating in the distension. The parameter β characterizes the elastic chains in the manner similar to Young's modulus. The notation L^{-1} is the inverse Langevin function. The changes in the constants of the equation are correlated with the growth curve and the incidence of disease in the rats.

Biological studies with 1-methylestrone. J. H. LEATHEM and MARY LEE CLARY (by invitation). *Rutgers Univ., New Brunswick, N. J., and Ciba Pharmaceutical Products, Summit, N. J.* Introduction of a methyl group into the aromatic ring of estradiol is reported to abolish its activity. Tests involving the effect of methylating estrone are here presented. 1-methylestrone failed to stimulate uterine weight or histology in immature rats in 48 hours after the single subcutaneous injection of as much as 2.0 mg. or after 10 daily injections of 0.25 mg. Furthermore, 1.0 mg. of 1-methylestrone did not alter the uterine stimulating action of concomitantly injected estradiol benzoate (2 μg.) when both steroids acted over 48 hours. The estrogenic action of 0.01 mg. of estradiol benzoate injected for 10 days was not influenced by the simultaneous administration of 0.25 mg. of 1-methylestrone in immature female rats. A single injection of 1.0 mg. did not influence seminal vesicle weight of immature male rats in 72 hours nor did it interfere with the action of 0.25 mg. of testosterone propionate. Administration of 0.5 mg. of 1-methylestrone daily for 10 days to 22 day old male rats failed to influence body weight increase or to alter the weights of the pituitary, adrenal, thymus, kidney, testis, seminal vesicle or ventral prostate. The gonadotrophic hormone content of the pituitary was normal. Thus the estrogenic action of estrone is greatly reduced or abolished by the introduction of a methyl group.

Plasma and liver protein concentrations in adrenalectomized and in thiouracil-fed adrenalectomized rats. J. H. LEATHEM. *Bureau of Biological Research and Dept. of Zoology, Rutgers Univ., New Brunswick, N. J.* Adrenalectomized adult male rats maintained for 20 days on 1% NaCl and pair-fed normals have comparable plasma protein levels although plasma albumin concentrations are subnormal, a reflection of subnormal food intake. Desoxycorticosterone acetate (Percorten, Ciba) (DCA) at 0.5 mg. daily permitted normal food intake and growth in adrenalectomized rats. Pair-fed controls exhibited normal plasma protein concentrations but the operated rat's plasma albumin level was not improved above that of a salt maintained rat due to either hemodilution or hormone deficiency. Hypothyroidism increases plasma glo-

bulin levels and thiouracil increases liver protein. Hypothyroidism in adrenalectomized rats maintained with NaCl or with DCA was studied by feeding 0.25% thiouracil for 20 days and comparing results with pair fed adrenalectomized and normal rats. Food intake was reduced by thiouracil with body weight loss resulting. Total plasma protein and plasma globulin levels increased following thiouracil. NPN was also higher whereas hematocrit decreased. Thyroid and liver weights were significantly above those of controls and analysis of the liver revealed a normal water content but a significant increase in total and relative amounts of liver protein in thiouracil fed rats. Adrenalectomized control rats maintained liver protein as well as pair fed normal rats. Thus, adrenalectomy does not alter the action of thiouracil on plasma and liver proteins.

Predisposition to vasodepressor properties of morphine induced by hemorrhagic hypotension. RICHARD E. LEE (by invitation) and BENJAMIN W. ZWEIFACH. *Dept. of Pharmacology, Syracuse Medical College, Syracuse, N. Y. and Dept. of Medicine, New York Hospital, New York City.* Several investigations concerning the action of morphine in hemorrhagic shock have not shown any consistent notable vasodepressor action. The agent was administered either prior to hemorrhage or during the early phases of a prolonged hypotension. Neither situation adequately duplicates the usual clinical sequence of events. In this study the morphine was administered following restoration of normal blood pressures by blood transfusion after a prolonged hemorrhagic hypotension; as well as prior to and during early 'shock.' Based on blood pressure recordings and direct visual observation of omental peripheral blood vessels, usual doses of morphine (1–3 mg/kg. intravenously) in unhemorrhaged dogs produce no significant or persistent vasomotor disturbances. When given early in hemorrhagic shock (1–2 hr. after initial bleeding) morphine administration is followed by a transient fall in blood pressure, accompanied by temporary vasodilatation with slowing of peripheral blood flow. In spite of restoring normal blood pressure by transfusion, after prolonged hemorrhagic shock, morphine in these doses is followed in all instances by a sharp severe fall in blood pressure. This coincides with observed arteriolar dilatation, depression of arteriolar reaction to topically applied epinephrine, and a severe slowing of peripheral blood flow, with stagnation occurring in the venular components of the capillary bed. Although immediate further transfusions were given each animal, 3 of the 12 dogs died in profound peripheral vascular collapse within 15 minutes after receiving morphine.

Physiological effects of bacterial polysaccharides upon the cardio-vascular and neuro-muscular systems. CHESTER E. LEESE and K. VIRGINIA GREENE (by invitation). *Dept. of Physiology, School of Medicine, George Washington Univ., Washington, D. C.* Rabbits were given doses of polysaccharide from 2 to 25 gamma/kg. Most of the animals showed a strong pyrogenic response associated with the usual maladjustments. Many animals lost the use of their body-righting reflexes. Lethal doses produced convulsive seizures, urination, defecation, and vomiting preceding death. Cats were less affected by the dosage level effective in rabbits. Pyrogenic reaction was noted before decerebration. Decerebration was often fatal when the animal was under the influence of the drug. If the drug was withheld until the animal recovered from decerebration, it tolerated a higher dosage without pyrogenic response. The cat showed lowering of arterial blood pressure, and an increased venous pressure. Both electrical and chemical stimulation of the vascular reflexes indicated a loss in the controlling power of the autonomic nervous system, or a lesser ability of smooth muscle to accept the stimuli. The vagus, splanchnic, and cardiosympathetic nerves required an increased strength of stimulus under polysaccharide influence. Assays with adrenalin, acetylcholine, pitressin, and histamine pointed to a loss in nervous response, rather than a decrease in the ability of smooth muscle to accept stimulation. Turtles perfused with polysaccharide showed cardiac changes. First to appear was an occasional dropped beat, followed by the various stages of heart block, and finally, complete dissociation. Tremendous dilation of both auricles and ventricles was noted. There occurs a progressive loss in the effectiveness of adrenalin or acetylcholine when added to the perfusate. Stimulation of the vagus also becomes less effective.

Food packages as a supplement to the German nutrition. G. LEHMANN (introduced by D. B. DILL). *Max Planck-Inst. fuer Arbeitsphysiologie, Dortmund, Germany.* The German nutrition of a man doing no heavy work reaches 1850 cal. per day. This includes 47 gm. of protein, of which only 11 gm. are of animal origin, and about 20 gm. of fat. A food parcel raising the nutrition of a family of 4 for one week to a normal level, therefore, should contain foodstuffs rich in protein and fat, for instance the following: 2.5 lb. of corned beef, 1 lb. of whole milk powder, 1 lb. of cheese, 0.5 lb. of powdered egg, and 1 lb. of margarine. Instead of corned beef, dried and smoked meat could be used. In contrast to the view of some nutritionists, the housewife will not take soy beans or other legumes as a valuable substitute for meat. The German housewife is not fond of pre-cooked meals but

prefers basic foodstuffs. The German nutrition is very rich in carbohydrates. Therefore, foodstuffs that are predominately carbohydrate should be avoided. Chocolate and cocoa are very welcomed and useful. Coffee and tea are important because of their stimulating effect. The addition of fruit juices marmalade, sweets, spices, etc. is less a matter of nutrition than a matter of making people happier. We are wrong in looking upon the question of food parcels from the point of view of the scientist only. Apart from raising the German nutrition to a normal level, they have the ideal task of intensifying friendship and understanding between men and nations.

Central hyperglycemic action of 1- (3,4-dihydroxyphenyl) 2-isopropylaminoethanol (isuprel). A. LEIMDORFER. *Dept. of Pharmacology, Loyola Univ., Chicago, Ill.* In dogs, under nembutal, the effects of intravenous (i.v.) and intracisternal (i.c.) injection of isuprel on blood-sugar, blood pressure, and electrocardiogram were studied. Nembutal itself produced no changes in blood sugar. After i.v. isuprel, 75 micrograms/kg., only a small, short lasting increase in blood-sugar occurred (onset about 15 minutes after injection, highest increase 40 mg per 100 ml.). The preinjection level was reached 45 to 60 minutes afrer injection. In contrast, after i.c. injection of the same amount, a much greater and long lasting increase in blood-sugar was found (onset about 15 minutes after the injection), the blood-sugar continued to rise for entire period of the observation (3½ hours), at that time it had risen to 180 mg. per 100 ml.

After i.v. isuprel, a marked fall in blood pressure, a marked tachycardia and ah extremely deep depression of the S-T segments were recorded. These changes were much smaller after i.c. isuprel. There seemed to be no direct relation between changes in blood-sugar and those in blood pressure and heart rate. Of importance might be the fact that after i.c. isuprel the blood-sugar was continuously rising whereas the changes in blood pressure and electrocardiogram were approaching the pre-injection levels. This fact and the particular blood-sugar curve after i.c. isuprel in contrast to that following i.v. isuprel suggest that the action of i.c. injection of isuprel in raising blood-sugar implies action on structures (centers) bathed by cerebrospinal fluid. These observations about isuprel which has an action like sympathin I are in conformity with our previous findings.

A method for collecting end-expiratory air from the oral cavity. GERSON LESSER, MORTON GALDSTON, and MARYLIN PRUSS (introduced by J. MURRAY STEELE). *Research Service, Third New York Univ. Medical Division, Goldwater Memorial Hospital, New York City.* In elderly or diseased patients it has often been impossible to collect alveolar air samples by the Haldane-Priestley method due to their inability to cooperate in performance of the necessary respiratory maneuvers. To overcome this difficulty and to maintain an undisturbed respiratory pattern, a simple, inexpensive method for collecting end-expiratory samples is being studied. Air samples are drawn at the end of a usual expiration through a narrow, semi-rigid tube, threaded through a rubber mouthpiece with its proximal open end 4–5 cm. within the oral cavity. The other end of the tube is connected to a small 3-way stopcock which is attached to the end of an evacuated mercury gas sample bottle. The stopcock permits washing out the tube with expired air just prior to collection of the end-expiratory sample. Preliminary evaluation of 40 observations in 4 young, healthy subjects suggests that for each individual there is a consistent relationship between the composition of end-expiratory air and alveolar air (Haldane-Priestley, end-expiratory). The pCO_2 was always lower (individual means of $\Delta\, pCO_2$ were 1.6 to 4.1 mm. Hg), and pO_2 was always higher (individual means of $\Delta\, pO_2$ were 2.4 to 7.9 mm. Hg) in end-expiratory than in alveolar air. In any given subject the greatest variation of a single observation from his mean was 3.5 mm. Hg pCO_2 and 6.4 mm. Hg pO_2 for end-expiratory air (26 observations); and 1.1 mm. Hg pCO_2 and 2.7 mm. pO_2 for alveolar air (14 observations).

Analysis of the electrocardiographic pattern produced by injection of epinephrine and nor-epinephrine in cats. EUGENE LEPESCHKIN and W. RAAB (introduced by F. J. SICHEL). *Division of Exptl. Medicine, Univ. of Vermont College of Medicine, Burlington, Vt.* Two to 6 seconds after intravenous injection of 20–40 micrograms epinephrine or nor-epinephrine in 4 cc. Ringer, a transient elevation of the T-wave in unipolar precordial or direct leads from both ventricles is observed. This elevation lasts 2–7 seconds, appears on injection of corresponding amounts of plain Ringer, and is due to cooling of the subendocardial ventricular muscle layers. The subsequent changes are due to the epinephrine or nor-epinephrine proper. Ten to 15 seconds after injection T becomes depressed over the right ventricle and elevated anew over the left ventricle (Phase I). During Phase II (until the 40th–50th second), T is inverted over the right ventricle, flattened (sometimes inverted) over the left ventricle. Phase III extends until the 120th-160th second and is characterized by marked elevation of T over both ventricles. In some cats T may again become temporarily inverted over the left ventricle between the 300th–400th seconds (Phase IV). These changes of the T-wave are independent of the changes of the heart rate. They are not due to blood pressure elevation (increased heart work), as they appear even if this elevation is abolished or reversed through previous administration of certain sympatholytic drugs (933F, SY28, DHE180,

Priscol). Other sympatholytic drugs (DHE 45, SY30) inhibit both the hypertensive reaction and the electrocardiographic changes. The latter cannot be due to direct action of epinephrine on subendocardial muscle layers of the right ventricle, as they appear in similar sequence and magnitude on injection of epinephrine into the left auricle.

Lecithinase activity of epiphyseal cartilage of the foetal pig. MILTON D. LEVINE and RICHARD H. FOLLIS, JR. (introduced by JOHN EAGER HOWARD). *Depts. of Medicine and Pathology, Johns Hopkins Univ., Baltimore, Md.* Lecithinase of the type described by Macfarlane and King (*Biochem. J.* 35: 884, 1941) has been demonstrated in the epiphyseal cartilage of foetal pigs. Homogenates of such cartilage, when incubated with lecithin suspension substrates in the presence of Ca^{++}, results in the liberation of neutral fat and free choline while the phosphorus of the substrate goes from the fat soluble to the water soluble state. The mechanism is thought to be as follows: Lecithinase splits lecithin into phosphocholine and neutral fat. Phosphocholine is a substrate for phosphatase; hence we have a hitherto undescribed source of phosphate ion. Lecithin and the appearance of neutral fat in cartilage will be discussed.

Nature of the action of insulin on the level of serum inorganic phosphate. R. LEVINE, S. D. LOUBE (by invitation) and H. F. WEISBERG (by invitation). *Dept. of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* It is well known that the administration of insulin, glucose or adrenaline causes a fall in the level of inorganic phosphate of the serum. We have previously shown (*Am. J. Physiol.* 134: 40, 1941) that no decrease in serum phosphate occurs after the administration of glucose or adrenaline to depancreatized dogs, indicating that the phosphate lowering effect of these substances is mediated by insulin. It was then concluded that the fall in serum phosphate is one of the direct actions of insulin. The rate of entry of fructose into tissues, as measured by the rate of disposal of intravenous fructose, is not diminished by the complete absence of insulin (pancreatectomy) (*Federation Proc.* 6: 21, 1947). We have, therefore, determined the effect of intravenous fructose on the serum phosphate level of untreated depancreatized dogs. In 9 of 10 animals there was a significant drop in inorganic phosphate of the serum following fructose administration. Under similar circumstances intravenous glucose does not affect the phosphate level. It is, therefore, concluded that the fall in serum phosphate is an expression of the rate at which a hexose enters the tissues. Insulin enhances this rate for glucose. Fructose, which enters tissues at a rapid rate even in the complete absence of insulin, causes a phosphate fall independent of the hormone. We can therefore no longer consider the fall in serum phosphate as a direct action of insulin. The only presently proven primary action of insulin is the increase in the rate of entry of glucose into tissue, specifically skeletal muscle.

Three dimensional electrocardiography. RAPHAEL B. LEVINE (by invitation) and OTTO H. SCHMITT. *Depts. of Zoology and Physics, Univ. of Minnesota, Minneapolis, Minn.* Much of the difficulty in the interpretation of electrocardiograms lies in correlating records of the several different potential variations which occur simultaneously. If it were possible to combine all of the information contained in the numerous individual-lead electrocardiograms into a single interpretable pattern, compactness would be obtained with no loss in generality. The 'vector cardiogram' of Wilson and Johnston is an approach in two dimensions to this ideal. Development of the 3-dimensional cathode ray oscilloscope has made possible the extension of electrocardiographic presentation to three dimensions. This instrument is capable of utilizing three electrical signals in such a way as to form a picture which to the eyes is a stereoscopically correct perspective pattern in space. An application of such an oscilloscope which immediately suggests itself is the presentation of a true 3-dimensional vector electro-cardiogram. This requires development of orthogonal leads or their electrical equivalent. In practice, it may prove entirely satisfactory to present data from conventional non-orthogonal leads in orthogonal form. As a further improvement, a computer currently under development will permit rotation and translation at will of any three-dimensional display to the angle and range most advantageous for viewing. These 3-dimensional displays can readily be photographed on standard film for stereoscopic viewing, and lend themselves well to the preparation of wire models.

Fat metabolism in Alaskan Eskimos. VICTOR E. LEVINE and CHARLES G. WILBER. *Arctic Research Laby., Office of Naval Research, Point Barrow, Alaska.* The blood sera of Point Barrow Eskimos (adults and children of both sexes) were taken for estimation of various lipid components. To date 50 analyses have been completed. Mean values are as follows:

LIPID COMPONENT	ALASKAN ESKIMOS	CANADIAN ESKIMOS (CORCORAN AND RABINOWITCH)
	mg/100 cc.	*mg/100 cc.*
Total fatty acids	701.3	555
Total cholesterol	221.6	141
Free cholesterol	124.9	46
Ester cholesterol	96.7	98
Lipid phosphorus	14.2	—
Phospholipids	355.0	164

The following were calculated: cholesterol/lipid phosphorus, 21.4; free cholesterol/total cholesterol, 0.56; cholesterol/fatty acid, 0.31. Our results compared with normal values for whites are high. The blood was taken late in August and early in September 1948, during which time walrus and seal were plentiful. The Eskimos were living close to their native diet. The values may, therefore, reflect a pronounced 'alimentary fat tide' due to the ingestion of fat-rich meals. Our findings are not in accord with those of Corcoran and Rabinowitch (*Biochem. J.* 31: 343, 1937) who found low lipid values in the bloods of 27 Canadian Eskimos taken at random. They did, however, report higher lipid values for natives on a meat diet compared to those for natives on a mixed diet. Under field conditions in the Arctic it is extremely difficult to collect blood in true fasting condition. Our results reflect a high fat intake, while those of the Canadian investigators may reflect a low fat intake or even a diet close to a starvation level.

Measurement of the intracellular cation in the dog. MARVIN F. LEVITT and MARIO GAUDINO (introduced by HOMER W. SMITH). *Dept. of Physiology, New York Univ. College of Medicine, New York City.* The measurement of the intracellular concentration of any cation in the living animal requires the determination of the volume of intracellular fluid and of the total intracellular cation. In our present experiments the intracellular fluid was calculated as the difference between total body water (D_2O space) and extracellular fluid (inulin space, *Proc. Soc. Exptl. Biol. & Med.* 68: 507, 1948). The total amount of body cation (Na or K) was measured by *in vivo* dilution of Na^{24} and K^{42}. A known amount of isotope was injected intravenously and the ratio of isotope to total cation in the plasma was followed until equilibrium was attained. Equilibrium requires 3 hours for NA^{24} (Kaltreider et al. *J. Exptl. Med.* 74: 569, 1941), and, according to our present observations, 9 hours for K^{42} in the dog. Observations with carefully purified K^{42} indicate that this cation penetrates tissues so rapidly that 85% of equilibrium is reached within 3 hours. At equilibrium, the counts retained in the body (considering urinary loss) divided by the total counts per mEq. of Na or K in the plasma, gives the total Na or K in the animal. In 2 dogs the average values were:

ELECTROLYTE CONCENTRATION (MEQ/L.)

	Na	K	Total
Extracellular (plasma)	150	4	154
Intracellular	34	111	145

Inhibition of a proteolytic enzyme system of blood. JESSICA H. LEWIS (by invitation) and JOHN H. FERGUSON. *Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N. C.* In certain human pathological conditions such as shock, anoxia, and severe liver disease, the phenomenon of liquefaction of a formed blood clot (fibrinolysis) has occasionally been observed. Normal serum contains no active lysin, the enzyme causing fibrinolysis, but does contain large amounts of the inactive precursor, prolysin, and a potent inhibitor, antilysin. The present report, dealing with certain reactions of antilysin, is part of a series of studies by which we are endeavoring to shed some light upon the mechanism of fibrinolysis in various human disorders and hemorrhagic shock in dogs. Standard curves are presented for the determination of potency of lysins partially purified from dog, human, and bovine sera, as measured by the lysis of a standard fibrin clot. The three curves are apparently identical. There is no detectible species specificity of each antilysin for its homologous lysin, as tested by the antilysin-assay method here presented. Serum antilysins do differ in important particulars from certain purified proteolytic enzyme inhibitors isolated from soybean, navy bean, and pancreas. Contrary to the suggestion that proteolytic enzymes may be inhibited by the products of protein digestion, it is shown that serum lysin is not inhibited by the products of fibrinolysis. Attempts were made to study the nature of the enzyme-inhibitor complex. It could not be split by differential heat destruction of the enzyme nor by chloroform treatment which has long been known to remove inhibitor from such prolysin-antilysin mixtures as serum.

Studies on lipoprotein in dog serum. LENA A. LEWIS and IRVINE H. PAGE. *Research Division and the Bunts Inst. of the Cleveland Clinic Foundation, Cleveland, Ohio.* Four protein peaks can be demonstrated by ultracentrifugation of dog serum previously diluted and dialyzed against 1.0, 1.5 or 2.0 M NaCl. They are similar to those described by Pedersen for human serum. The peak with the slowest sedimentation rate is not demonstrated at salt concentrations of 0.6 M NaCl or less, and is only poorly resolved with 0.75 M NaCl. Using 2.0 M NaCl—2% dog serum protein solution, prolonged centrifugation in the preparative rotor was done. The top layer which had a 'milky appearance' was removed and studied by electrophoresis and ultracentrifugation. With phosphate buffer *p*H 7.8, ionic strength 0.16 μ, two fractions were demonstrated by electrophoresis. One had a mobility similar to that of serum albumin (dog) and represented 57% of the total area. The slower moving component had a mobility similar to that of α_2-globulin (dog) and represented 43%. The albumin in the dog

serum was 47.9% and the α_2-globulin 6.2% of its total protein. The slow sedimenting lipoprotein of human serum has the electrophoretic mobility of β_1-globulin. In the ultracentrifuge two peaks were demonstrated in the lipoprotein concentrate when salt concentrations of 1.0 and 1.5 M NaCl were used. The faster sedimenting component had a sedimentation rate similar to serum albumin and represented 62% of the total area. The slower sedimenting fraction represented 38%. A slow sedimenting lipoprotein with the electrophoretic mobility of α_2-globulin can be demonstrated and concentrated from dog serum by ultracentrifugation when salt concentrations between 1.0 and 2.0 M NaCl are used.

Metabolism of lactate by the isolated dog gastrocnemius studied with α,β- and carboxyl-labeled lactate. NATHAN LIFSON, AKIRA OMACHI (by invitation), and SHIRLEY L. MICHEL (by invitation). *Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* The isolated gastrocnemius of a nembutalized dog was perfused at physiological pressures and temperatures by means of a closed, pump-oxygenator circuit, the animal's own heparinized blood serving as the perfusion fluid. The muscle was either at rest or was stimulated via the sciatic nerve with maximal single shocks at a frequency of 2/sec. for 2–3 hours. Either α,β- or carboxyl-labeled racemic C^{13}—sodium lactate was administered to increase the blood level by approximately 1.4 mM/100 cc. In 3 experiments with stimulated muscle it could be calculated from

$$\frac{\text{total mM } CO_2}{3} \times \frac{\text{atom \% } C^{13} \text{ excess in respiratory } CO_2}{\text{atom \% } C^{13} \text{ excess in administered lactate}}$$

that 0.56–0.94 mM/100 gm. of muscle/hr. of administered lactate was converted torespiratory CO_2. The addition of non-isotopic acetate or succinate to give blood levels of about 0.8 mM/100 cc. reduced these values by at least 50% . In these latter experiments acetate, succinate, and lactate were recovered from blood and/or muscle and analyzed for C^{13}. Resting muscles, to which was administered glucose with or without insulin, also converted administered lactate to respiratory CO_2 but at slower rates than did stimulated muscles. In the stimulation experiments the glycogen content of the perfused muscle decreased in comparison with that of the corresponding unperfused muscle of the other side. In the experiments with resting muscles, the glycogen content increased when insulin was administered. However, in no instance was definite significant excess C^{13} found in the muscle glycogen. From the above data it is concluded that: *1*) lactate is converted to CO_2 by both resting and active mammalian skeletal muscle; *2*) the presence of other substrates (acetate and succinate) decreases the rate of conversion of administered lactate to CO_2; and *3*) unequivocal evidence for the conversion of lactate to glycogen in either contracting or resting muscle was not obtained.

Further studies on the single muscle fiber membrane potential. G. LING and R. W. GERARD. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* Studies on the membrane potential of frog sartorious fibers, singly impaled with a capillary electrode (Ling, Ling and Gerard), have been extended. The A potential fraction, between 54 and 78 mV, has been shown to parallel fairly well the actual CrP content under the influence of tetanization, methylene blue, cyanide, azide, hydroxylamine, iodoacetate alone or with lactate. The B potential, between 0 and 54 mV, is not directly dependent on metabolism but varies with K^+; increased K^+ lowers the plateau between A and B potentials which appears when the A potential has been abolished by some of the above conditions. Continuous irrigation of the muscle surface is necessary to avoid accumulation of leaking K^+. Thus, in unstirred K-free solutions, the potential rises irregularly, seldom above 90 mV; in flowing ones, it can rise to 108 mV. Also, the fall in N_2 is less in flowing than still solutions, and that in IAA starts later. In general, potential varies inversely as log of external K^+ concentration (slope = 44 mV per tenfold change) to −7.0 mV at 4 times isotonic K^+. The slope of the K^+-potential curve is seemingly altered by addition of Ba^{++} but not by Ca^{++}. Exposure to CO_2, in contrast to nerve, lowers the normal value by 5 to 10 mV during 1 to 2 hours; return in air is complete in 1 hour. In 20% or 100% CO_2, alike, the A potential is lost in half to one hour; returns in a few minutes. Absence of K^+ from the irrigation fluid does not alter the size of the CO_2 depression. Conclusions can be drawn from these data as to the nature of the membrane.

Effect of temperature on the membrane potential of the frog sartorius muscle. G. LING and J. W. WOODBURY (introduced by R. W. GERARD). *Dept. of Physiology, Univ. of Chicago, Chicago, Ill., and Dept. of Physiology, Univ. of Utah, Salt Lake City, Utah.* Variations of membrane potential with temperature were measured in the sartorius muscle of *Rana pipiens* by inserting a micro-electrode directly into a single fiber. Four to eight potential measurements were made on each muscle at from 4 to 6 different temperatures between 3° and 41°C. on a total of 28 muscles. The muscles were allowed to equilibrate at each temperature for at least 20

minutes. Temperature was progressively increased in 14 experiments, decreased in ten, and first increased then decreased in four. The averaged data from 8 muscles (temperature increasing) are tabulated below:

T° C.	5	15	20	23	30	37	39	40	40.5	41
V mv.	71	80	83	85	87	85	83	80	71	63

For progressively increasing temperature from 5°C. to 25°C. the relation between log voltage and reciprocal of absolute temperature was approximately linear with a Q_{10} of 1.1 (activation energy 1700 calories). The mean Q_{10} for all muscles was 1.08 (1400 calories). Membrane potential reached a peak at approximately 30°C. and fell precipitously and irreversibly above 35°C., in association with contracture. The low temperature co-efficient may possibly represent the difference between energies of activation for penetration of the membrane by positive and negative ions produced by metabolic processes.

A modified procedure for inulin determination in plasma and urine. J. MAXWELL LITTLE, JUNE HARRIS (by invitation) and LEWIS FRANKLIN (by invitation). *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.* Oxidative destruction of glucose by heating in the presence of NaOH has been utilized to replace the usual destruction by yeasting (Selkurt: *Methods in Medical Research 1:* 197, The Year Book Publishers, Inc., Chicago, 1948). The effects of NaOH concentration and duration of heating in boiling water upon glucose and inulin were studied. Incomplete glucose destruction (with 50–300 mg.% solutions) or production of an absorbing intermediate compound was indicated by light transmissions of 93–99% when compared with a water blank set on 100%. This residual absorption was almost completely rectified by the addition of 1.0 cc. of 100 mg.% glucose to the blank, which was set on 100%. *Procedure:* plasma, or diluted urine, containing 5–30 mg.% inulin, is precipitated with $CdSO_4$ and NaOH after the addition of 1.0 cc. of 100 mg.% glucose solution. Shake the mixture vigorously for 10 minutes, centrifuge, and filter through washed cotton. To 4.0 cc. of filtrate, in a large test tube, add 1.0 cc. of 4.0 N NaOH, cover with marbles and immerse in boiling water for 10 minutes. Cool, add 10 cc. of diphenylamine reagent and immerse in boiling water for 30 minutes. Cool the tubes and read in an Evelyn colorimeter using filter 620. Blank tubes contain plasma or urine without inulin. Mean recoveries and standard deviations with theoretical concentrations of 5, 10, 15, 20, and 30 mg.% inulin for plasma were respectively: 5.10 ± 0.293, 9.93 ± 0.357, 15.21 ± 0.664, 20.25 ± 0.846, and 30.53 ± 0.700; for urine: 4.98 ± 0.235, 9.78 ± 0.272, 14.94 ± 0.224, 20.16 ± 0.479, and 30.49 ± 0.523.

Atrial hemodynamics in the presence of experimentally produced interatrial septal defects. R. C. LITTLE (by invitation), J. G. HAWLEY (by invitation) and D. F. OPDYKE. *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, Ohio.* Interatrial septal defects were produced in open chest dogs. Right and left atrial pressures were recorded simultaneously. The results of experimental alteration of atrial pressures before and after producing the defect are compared. The left-to-right pressure difference between the atria which was observed previously in similar preparations without septal defects persists after producing the defect, although the differential appears to be decreased. Rapid saline infusion elevates both left and right atrial pressures in the presence of an interatrial defect, but does not appear to increase left pressure as effectively as in the control. However, right pressure does not exceed left, although the two pressures tend to equalize after a considerable volume of infusion.

As in the control series, positive pressure inflation of the lungs (open-chest) results in an elevation of both right and left atrial pressure, but right increases more than left with a subsequent reduction of the pressure differential. Persistence of a pressure differential is probably due to a difference between the volume-elasticity characteristics of the atria. At equilibrium the volume flow into each atrium is equal. Since the left atrium has the greater volume-elasticity coefficient, the pressure will be greater than that in the right atrium. Experiments with a physical model indicate that this is a major factor in maintaining the pressure difference.

Effects of breathing CO_2 on blood pressure and renal circulation in normotensive and hypertensive subjects. W. J. LITTLE, J. W. AVERA and S. W. HOOBLER (introduced by M. H. SEEVERS). *Dept. of Medicine, Univ. of Michigan Hospital, Ann Arbor, Mich.* The mean blood pressure increased 21% in 6 normotensive subjects and 16% in 5 hypertensive subjects during inhalation of a gas mixture containing 10% CO_2 and 90% O_2. This rise was theoretically due to increased vasomotor tone, but was probably in part the result of an increase in cardiac output, since it occurred after caudal anesthesia had been established to dorsal 3. The procedure was also associated usually with

renal vasoconstriction, while control studies utilizing hyperventilation of room air and rebreathing 100% oxygen had little effect on renal circulation. The following table summarizes observations with 6 normals and 5 hypertensives, showing the average (and range) percentage change during CO_2 inhalation.

	NORMOTENSIVES	HYPERTENSIVES
RPF.....	−13% (0 to −39%)	−23% (−9 to −66%)
RR.....	+46% (+7 to +110%)	+75% (+23 to +228%)
GFR.....	−2% (−31 to +16%)	−12% (−59 to +12%)
FF.....	+10% (0 to +17%)	+17% (+9 to +25%)

RPF is renal plasma flow (PAH); RR is renal resistance (Mean BP/RPF); GFR is glomerular filtration rate (mannitol); and FF is filtration fraction (GFR/RPF). The changes were usually abrupt in onset and offset, occurring only during the 10 min. period of CO_2 breathing. The failure of a 'rebound' phenomenon to occur in the ensuing urine collection periods excluded the possibility that the changes were due to retention of urine in the renal pelvis. The evidence seems to indicate that the renal vasoconstriction observed in the human subject, during inhalation of 10% CO_2, is primarily the result of an increase in neurogenic vasoconstrictor activity.

Post-tetanic potentiation of presynaptic actions in the spinal cord. DAVID P. C. LLOYD. *Labys. of The Rockefeller Inst. for Medical Research, New York City.* Monosynaptic reflexes of a given muscle are greatly increased forseveral minutes following brief tetanization of the nerve afferent for that muscle, the phenomenon resembling that studied in ganglia by Larrabee and Bronk, in frog by Bremer and Kleyntjens. Tetanizing afferent pathways, from other muscles or skin, that converge, directly or after internuncial relay, with the monosynaptic pathway of the given muscle may influence transmission therein for 15 seconds, but prolonged potentiation is not realized. Furthermore, penetration into motoneuron somata of antidromic volleys, depressed for several seconds following presynaptic tetani, is not further influenced. If, however, the nerves of two muscles, synergists or antagonists within a myotatic unit, are stimulated by single shocks so related in time that the volley in one nerve, through monosynaptic connection, facilitates or inhibits the monosynaptic reflex elicited by stimulation of the other nerve, then, following tetanization of the first-mentioned nerve, its conditioning action, facilitatory or inhibitory, upon the test reflex, is enhanced for several minutes, the test reflex in isolation remaining unaffected. Now, since any activity evoked by means other than stimulation of the tetanized afferent fibers is not potentiated, and since the known synaptic actions of those afferent fibers all are, it follows that the process underlying post-tetanic potentiation must be sought in the rhythmically stimulated afferent fibers themselves. Recordings from the intramedullary afferent fibers of post-tetanically increased impulses displaying subnormal velocity suggest that hyperpolarization (positive after-potential) by increasing in size the presynaptic impulses causes them to be more effective synaptic agents.

Effect of sham-feeding on gastric motor activity of the dog. STANLEY H. LORBER (by invitation), S. A. KOMAROV and HARRY SHAY. *Samuel S. Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Penna.* The effect of sham-feeding on the gastric motor activity of a dog with an esophagotomy and gastrostomy has been studied by a balloon-kymograph technique. A balloon was introduced into the gastric antrum through the gastrostomy opening and, after a 30–60 minute period of observation, the dog was sham fed. Results observed were of two types and depended upon the basal motor activity. In the active stomach, sham-feeding abolished peristaltic waves and diminished or abolished tonus waves. The diastole of tonus activity was not altered appreciably. The above effects occurred promptly at the start of sham-feeding and preceded acid secretion by 5–7 minutes. The motor inhibition lasted for several hours. In the inactive stomach, sham-feeding resulted in the initiation of motor activity and in an increase in tonus. The waves induced were predominately of the tonus type but for a few minutes after sham-feeding peristaltic waves also occurred. The effects preceded or immediately followed the termination of sham-feeding. The results indicate that sham-feeding can induce or inhibit gastric motility depending upon the state of basal gastric activity.

Conversion of propionate carbon to liver glycogen in the intact rat, studied with C^{14},C^{13}-labeled propionate. VICTOR LORBER, MARGARET COOK (by invitation) and JULIEN BORDEAUX (by invitation). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.* Solutions of $C^{14}H_3C^{13}H_2COO$-Na and glucose were given to fasted rats by stomach tube. Resulting liver glycogen was isolated, hydrolyzed to glucose and the position of C^{14} and C^{13} in the glucose molecule determined (*J. Biol. Chem.* 159: 475, 1945). Prior experiments (*Federation Proc.* 6: 272, 1947);

Lorber, Lifson, Sakami, and Wood, unpublished) in which $CH_3C^{13}H_2COO$-Na and $C^{13}H_3CH_2COO$-Na were fed to different animals have indicated that isotope administered separately in the α and β carbons appears in both cases mainly in the 1,6 and 2,5 carbons of glucose from liver glycogen, and is equally distributed between these positions. In the present experiments in which it was possible to study the fate of isotope from both the α and β carbons of propionate simultaneously in the same animal, the above result has been confirmed. Following feeding of $CH_3C^{13}HOHCOO$-Na, the isotope is incompletely randomized between carbons 1,6 and 2,5 of the glucose from liver glycogen (Lorber, Lifson, Wood, and Sakami, *Proc. Am. Physiol. Soc.*, in press), positions 2,5 predominating. The results with propionate may therefore be interpreted to mean that for the conversion of propionate to pyruvate some pathway other than a direct conversion via α oxidation must exist.

Role of insulin in the metabolism of amino acids. WILLIAM D. LOTSPEICH. *Syracuse Univ. College of Medicine, Syracuse, N. Y.* It has been repeatedly demonstrated that the injection of insulin results in a fall in the plasma amino acid nitrogen. The best evidence to date indicates that amino acids are being synthesized into protein under the influence of insulin. In order to further clarify this problem the following study was undertaken. Normal adult female dogs, fasted for 12 hours, were injected intravenously with insulin (Iletin-Lilly) in doses of 2 U/kg. body weight. The whole blood levels of the natural forms of the 10 essential amino acids were followed for 1 hour thereafter. All amino acids were determined on tungstic acid filtrates of whole blood by microbiological assay techniques using *Streptococcus fecalis-R, Leuconostoc mesenteroides* and *Lactobacillus arabinosus* as the test organisms. It was found that the blood levels of all amino acids fell at different rates. Arranged in the order of decreasing extent of fall the amino acids were: leucine, lysine, isoleucine, valine, threonine, arginine, phenylalanine, histidine, methionine and tryptophane. It was further found that there existed a direct correlation between the quantity of each amino acid leaving the blood after insulin and the concentration of that same amino acid in dog skeletal muscle protein. This was taken as presumptive evidence that insulin plays a role in the synthesis of protein from circulating free amino acids.

Action of epinephrine on the pyloric sphincter as recorded by the pyloric inductograph. HORTENSE LOUCKES (by invitation), DANIEL A. BRODY (by invitation) and J. P. QUIGLEY. *Division of Physiology, Univ. of Tennessee, Memphis, Tenn.* We have employed a modified form of the pyloric inductograph of Brody and Quigley (*J. Lab. & Clin. Med.* 29: 3: 1944). By increasing the primary coil input from 60 to 1500 cycles per minute the 50 ohms resistence coils become more sensitive and much smaller coils have adequate sensitivity. The amplifier output has been rectified so that coil or sphincter movements are recorded as a line insead of a band. This facilitates interpretation of the records. Normal dogs, thoroughly trained to participate in the studies, were provided with two coils of 50 ohms resistance sutured at the pyloric sphincter diametrically opposite each other. The pyloric sphincter of the dog usually exhibited spontaneous rhythmic contractions having a 10–14 second cycle. This interval was not altered by the fasting or postprandial state but the contractions became more regular, of greater rmplitude and spontaneous periods of inactivity were rare when the stomach contained food. The intravenous injection of epinephrine (0.5–4 gamma/kg. completely abolished postprandial motility for 30 to 85 seconds respectively and depressed motility for an additional 10 to 60 seconds respectively. During the recovery period the frequency of sphincter contractions returned to the pre-injection rate. Epinephrine increased the sphincter tone and this increase was of longer duration (30 to 240 seconds) than the motor inhibition. The latent period of epinephrine effect ranged from 15 to 25 seconds. A reduction in pulse rate paralled the decreased sphincter motility. Doses less than 0.5 gamma/kg. epinephrine appeared to be subthreshold for they produced questionable inhibition of sphincter motility, no effect on tonus and a slight decrease in pulse rate.

Absorption of sulfamylon. E. W. MCCHESNEY, M. E. AUERBACH (by invitation) and H. W. ECKERT (by invitation). *Sterling-Winthrop Research Inst., Rensselaer, N. Y.* Studies on the absorption of Sulfamylon (homosulfanilamide, marfanil) have been retarded by the lack of a satisfactory method for its determination in blood. The method of Heideman and Rutledge (*J. Pharmacol. & Exptl. Therap.* 93: 451, 1948) is open to several criticisms, principally on the ground that the metabolic product, p-carboxybenzenesulfonamide, also absorbs light in the region 265–270 mμ, and absorbs it more strongly than Sulfamylon. An alternate method for the determination will be presented, based on the Ehrlich and Herter reaction for α-amino groups (*Z. physiol. Chem.* 41: 329, 1904). The method involves precipitation of the whole blood proteins with trichloroacetic acid, adjustment of the filtrate to pH 9, and reaction with β-naphthoquinone sulfonate. The yellow derivative is extracted with methyl n-amyl ketone, and is de-

termined in the photoelectric colorimeter using a 420 mμ filter. This method has been applied in studies of absorption of the drug. Dogs given 625 mg/kg. by intravenous infusion showed blood levels ranging from 15 to 80 mg.%, 30 minutes after the end of the infusion; the blood was essentially cleared of Sulfamylon in 5 hours. When the same dose was given orally, the peak blood levels ranged from 7 to 35 mg.%, 2 to 3 hours after medication. In human subjects an oral dose of about 110 mg/kg. gave peak blood levels of the order of 7 mg.%, 1 to 2 hours after medication.

Inhibition of the knee jerk from tendon spindles of crureus. G. P. McCouch, I. D. Deering (by invitation), W. B. Stewart and W. W. Chambers (by invitation). *Depts. of Physiology and Anatomy, Univ. of Pennsylvania, Philadelphia, Penna.* In decerebrate cats, the muscles of the lower extremity were silenced by denervation or tenotomy except for quadriceps and usually semitendinosus. The limb was fixed by drills and a pelvic clamp. Muscle tension was recorded isometrically and action potentials, from a bare needle through rectus and crureus. The knee jerk was elicited by a mechanical tapper, which stretched the muscle by raising the lever shank. Conditioning volleys, usually below and never significantly above threshold for direct or neuromyal response, were delivered through bare needles of which one passed through the patellar ligament, the other transversely through vastocrureus a few millimeters proximal to the tendon of crureus. Inhibition curves may be preceded by facilitation at intervals between conditioning and test stimuli up to 2 msec. This is followed by a descending limb, relatively constant in shape and duration, which reaches maximal depth within 8 or 9 msec. Toatl duration of inhibition is variable, being greater with more proximal electrode positions, which involve more receptors, than with distal positions, which engage fewer end organs. It ranges from 20 to 100 msec. Two factors may be postulated to account for the shape of the curve. The first is facilitation from the inevitable involvement of muscle spindles by the conditioning stimulus. The second results from the multisynaptic character of the inhibitory arc and its consequent afterdischarge.

Vascular changes in the warmed ear during oximetry. W. J. McCracken (introduced by J. K. W. Ferguson). *Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada.* A photoelectric oximeter developed by Dr. W. Paul employs amplification and meters such that fluctuations of blood content of the ear ('ear thickness') are observable with each pulse beat and amount to 0.25–1.5 ma. The reading with the ear compressed and bloodless is less than one ma., while fully warmed ears give readings of 9–15 ma., or about 8 ma more than unwarmed ears. Over and above fluctuations with the pulse, changes have been observed after *a*) compression of the ear (tissue anoxia for 3–5 min.), *b*) hyperventilation, *c*) anoxia by holding the breath, and *d*) breathing oxygen. After compression of the ear the current increased by 0–3 ma. in 19 subjects (av. 1 ma.) above the precompression reading, and returned to the precompression reading in 3–5 minutes. This is interpreted as reactive hyperemia which temporarily increased the blood content of the ear by an average of about 20%. Hyperventilation in 2 subjects proudced vascular constriction, as shown by a decreased current of about 1 ma. Subsequent anoxia by breath-holding without increased intrathoracic pressure caused an increased current of about 4 ma. above normal, indicating additional vasodilatation in the ear. Breathing O_2 (50, 75, and 99%) caused a decrease of current of 0–2 ma. in 19 subjects (av. 0.75 ma.). The magnitude of this vasoconstriction did not seem to vary with O_2% from 50–99%.

Experimental studies with psoriatic blood. David I. Macht. *Dept. of Pharmacology, Labys. of Sinai Hospital, Baltimore, Md.* For 25 years, the author has been employing plant-physiological methods for studying pharmacological properties of blood. In this way by testing 1% solutions of sera in plant physiological saline on root growth of *Lupinus albus* seedlings, the presence of toxic substances was demonstrated in menstrual blood and in the blood of pernicious anemia, pemphigus, leprosy, and trachoma. Numerous experiments with psoriasis blood however on normal seedlings gave readings exactly the same as with normal blood sera. Three years ago, the author began experiments on the action of drugs and sera on seedlings which had been previously vernalized. He found that ethylcarbamate was much more toxic for vernalized seedlings than for normal ones. Similarly it was discovered that psoriatic serum was toxic for vernalized seedlings but not for normal seedlings; all the other phytotoxic sera mentioned above did not show such a difference.

Vernalization (or 'yarovization') in these experiments was produced by chilling seedlings at 42°F in the dark (Miller, S. C. *Plant Physiology*, 1938). This finding in psoriasis is useful for diagnosis and also for the evaluation of therapeutic procedures. Thus exposure of psoriatic sera to x-rays *in vitro*, or to ultra-violet rays in quartz produce detoxification of psoriatic blood. These physical agents are employed with somd success in the treatment of that disease. Extracts of *Smilax* sarsaparilla also detoxify psoriatic serum in vitro; and that drug has

also been employed clinically (*New England J. Med.* 227: 128, 1942).

Physiological, pharmacological and biochemical studies on blood of animals in rage. DAVID I. MACHT and THOMAS HOFFMASTER (by invitation). *Dept. of Pharmacology, Labys. of Sinai Hospital, Baltimore, Md.* Six different experimental studies were made on the blood of rabbits and cats before and after enraging them: *1*) clotting time of whole blood measured by the Lee White method; *2*) prothrombin time estimated by Quick's method; *3*) blood sugar determined by microchemical methods; *4*) phytotoxic effects on root-growth of seedlings measured on *Lupinus albus* by Macht's method; *5*) reducing power of blood sera for methylene blue studied in a substratum of crushed vegetable cells by Thunberg's method; *6*) similar studies with serum on brain and muscle tissues, made by Thunberg's method.

Coagulation time of whole blood in rage is markedly reduced, as much as 50% or more. Prothrombin time is but slightly affected, the fluctuations being of little significance. Blood sugar is markedly increased for 6 hours or more. Phytotoxic properties of sera after rage are very striking and are exhibited by them even after refrigerating the blood specimens for 3 days. Control experiments with intravenous injections of epinephrinealso shorten coagulation time and produce hyperglycemia, but render the blood phytotoxic only when tested a few hours after drawing it; the phytotoxicity disappears, probably through oxidation, on standing several hours. Significant differences also are shown between the reducing power of the sera after rage and after injections of epinephrine, respectively. The results indicate that the blood changes in rage cannot be attributed entirely to adrenal effects, but involve other metabolic or endocrine disturbances.

A new chemical method for the determination of histamine in biological materials. FLOYD C. MCINTIRE, MURIEL SPROULL, and FRANCES B. WHITE (introduced by R. K. RICHARDS). *Dept. of Biochemistry, Abbott Research Labys., North Chicago, Ill.* The reaction between histamine and 2,4-dinitrofluorobenzene may now be used for the determination of histamine in biological materials. The histamine to be determined is purified by the cotton acid succinate method (McIntire, Roth, and Shaw, *J. Biol. Chem.* 170: 537, 1947). The cotton acid succinate eluate is not neutralized, but is evaporated to dryness. The dry residue is dissolved in a small amount of an alkaline buffer, an alcoholic solution of dinitrofluorobenzene is added and after 20 minutes at room temperature the solution is acidified. Under the conditions employed, histamine reacts with dinitrofluorobenzene to yield β-(4-imidazolyl-N-(2,4-dinitrophenyl) ethylamine. This histamine derivative is separated from the excess reagent and from a number of dinitrophenylamino compounds by a micro counter-current extraction procedure. Interference from histidine and diamino acids is eliminated by a second counter-current extraction under alkaline conditions. The quantity of histamine derivative is determined by measuring optical density at 358 mμ in a Beckman spectrophotometer. By employing microcells in the spectrophotometer it is possible to determine as little as 0.2 μg. of histamine with an accuracy of ±5%. The histamine derivative is very stable and the results are highly reproducible. In our application of this method to rabbit whole blood and plasma, the chemical method has given good agreement with bioassays. The method is particularly suited for the handling of a large number of samples at one time.

Changes in the amount of protamine sulfate required to re-establish *in vitro* clotting of heparinized blood in certain pathologic states. CHARLES L. MCKEEN, RICHARD M. ELGHAMMER and PETER V. MOULDER (introduced by J. GARROTT ALLEN). *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* The amount of protamine sulfate required to allow coagulation of standardly heparinized blood is constant in normal human beings and dogs. In certain human pathologic conditions, and in artificially produced toxic states in dogs, this protamine sulfate requirement increases. In humans, this phenomenon has been observed in leukemia, menorrhagia and in certain patients with coagulation disturbances, otherwise unclassified; it has also been seen following internal and external irradiation and aminopterin and nitrogen mustard therapy. It has been produced in dogs by total body irradiation, aminopterin and nitrogen mustard. In these instances of changed protamine requirements, clotting times, though usually mildly prolonged, are only occasionally normal and rarely incoagulable. This changed protamine requirement is independent of the thrombocyte count. Intravenous or intramuscular protamine sulfate and intravenous toluidine blue return the *in vitro* protamine sulfate requirement to, or toward normal.

Concurrent estimates of blood volume in rabbits by bleeding and dye methods. PAUL L. MCLAIN, C. H. WILLIAM RUHE (by invitation) and T. K. T. KRUSE. *Dept. of Physiology and Pharmacology, School of Medicine, Univ. of Pittsburgh, Pittsburgh, Pa.* In 20 rabbits, the blood volume by Guthrie's modified Welcker method averaged 126.2 ± 3.21 ml. or 4.28 ± 0.078% of the body weight. The animals were bled and simultaneously infused

with saline so as to reduce the circulating hemoglobin as much as possible before failure of the heart. Infusion was continued, with manual pumping of the heart, until the hemoglobin concentration of the effluent was well below 1% of the original. Finally, the limbs were massaged, all large vessels slashed, and the animal drained. All precautions were taken to insure that no significant amount of hemoglobin was left in the animal. In 15 of these animals, plasma volume was estimated concurrently by the dye T-1824. The line established by 3 points on the dye disappearance curve was extrapolated to zero time both rectilinearly and logarithmically. Blood volume was estimated from plasma volume and arterial hematocrit. Ordinary plotting yielded blood volumes 7.23 ± 0.29% of the body weight, logarithmic plotting 5.67 ± 0.18%. Estimates by the 2 dye methods differed significantly from each other and from those obtained by hemorrhage, and were much less consistent when related to body weight. The average plasma volume obtained by rectilinear plotting exceeded the average whole blood volume estimated by the bleeding technique. The discrepancies between the results of hemoglobin and dye procedures seem too large for explanation by reasonable differences between arterial and 'body' hematocrit, and suggest serious loss of dye from the circulating plasma shortly after injection.

Assay of FSH, ICSH, and luteotrophin in the hypophysectomized rat: clinical applications. WILLIAM O. MADDOCK (by invitation), EDWIN C. JUNGCK (by invitation), DEMETRIOS A. RIGAS (by invitation), and CARL G. HELLER. *Depts. of Physiology and Medicine, Univ. of Oregon Med. School, Portland, Oregon.* Current methods for assessing gonadotrophins in clinical materials usually indicate whether titers are greater or less than normal, but rarely indicate which of the 3 gonadotrophins are present or predominate. Purified FSH alone does not cause ovarian secretion. FSH plus ICSH stimulates ovarian secretion of estrogen, and the addition of luteotrophin stimulates progesterone secretion. This serves as a basis for the qualitative assay of a mixture of gonadotrophins. Thus, if an unknown extract causes estrogen elaboration from the ovary, FSH and ICSH must be present; if, in addition, progesterone secretion is produced, luteotrophin must also be present in the extract Estrogen and progesterone secretion can be determined by the effects of these hormones on the immature rat uterus. Alpha-estradiol, administered to castrate or intact immature rats, produced thin-walled, translucent uteri, uniformly ballooned with fluid. Doses of alpha-estradiol 1000 times an effective dose still produced thin-walled, translucent, fluid-filled uteri. Simultaneous administration of estradiol and progesterone produced thickwalled, opaque uteri, devoid of fluid. Administration of extracts containing predominantly FSH and ICSH to hypophysectomized rats with intact ovaries produced fluid-containing uteri. The same extracts plus luteotrophin produced stimulated uteri devoid of fluid. Administration of extracts of the urine of castrate and normal men have produced uteri devoid of fluid, similar to those produced by estrogen plus progesterone treatment, or by administration of extracts containing FSH, ICSH and luteotrophin. It is concluded that all 3 gonadotrophins are excreted by normal and castrated men.

Possible relationship between cinchophen ulceration of the stomach or duodenum and the choleretic response to cinchophen. D. F. MAGEE (by invitation), K. S. KIM (by invitation) and A. C. IVY. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Cinchophen in dogs is both ulcerogenic and choleretic, whereas in rabbits neither action is observed. The object of this investigation was to determine if this relationship existed in other species. Two sets of experiments were made using cats, rabbits, guinea pigs and rats. *1) Acute.* Under 'nembutal' anesthesia the common bile duct was cannulated, the gall bladder tied off and the half hourly bile output collected and measured. The differences in volume between the basal flows and those after 100 mg/kg. of intravenously administered cinchophen dissolved in N/10 sodium hydroxide were determined. As a test of reactability intravenous 'decholin' was given. The basal bile flow in cats was 0.5 ml/kg/hr. in guinea pigs 10 ml/kg/hr. Increases after cinchophen of 50% and 60% respectively were observed. The basal flow in rabbits was 4 ml/kg/hr. in rats 2 ml/kg/hr. No choleresis was observed after cinchophen, but it occurred after 'decholin.' *2) Feeding experiments.* Capsules containing 100 and 200 mg/kg. were administered daily. The rats were fed the same per kg. dose dissolved in N/10 sodium hydroxide by stomach tube. Equal quantities without cinchophen were given to the control rats. Consistent ulceration was produced only in the cat. Some rabbits and guinea pigs developed gastritis and duodenitis but no ulcers were histologically demonstrable. All the animals, except the rats, fed the sodium salt, died in from one to eight weeks of cinchophen feeding. There is apparently no relation between cinchophen induced choleresis and cinchophen ulcer.

Effect of blood composition on the metabolic activity of the perfused brain in the living cat. J. MAGNES, R. M. TAYLOR and A. GEIGER (Intro-

duced by H. GRUNDFEST). *Dept. of Psychiatry, College of Physicians and Surgeons, Columbia Univ., New York City.* In continuation of experiments on the metabolism of the perfused cat's brain, attempts were made to facilitate the identification of essential brain metabolites by simplifying the composition of the perfusion blood. Perfusion experiments were made with *1*) fresh, defibrinated, heparinized beef blood passed through an isolated cat liver for removal of vasoconstrictor substances; *2*) the same blood but dialyzed against 0.9% NaCl solution in the cold for 6 days, and reconstituted with electrolytes and glucose; *3*) exhaustively washed beef blood corpuscles suspended in a Ringer's solution containing 7% bovine serum albumin. All 3 preparations maintain brain functions. Preparation (*3*) shows no vasoconstrictor properties. Cerebral oxygen consumption and blood flow were highest with (*3*) and lowest with (*1*) with equally good functional activity of the brain. The rate of O_2 consumption of the brain is related to the functional activity of the brain as long as reflexes can be elicited, but the actual rate with (*1*) is between 4–6 ml. O_2 consumed by 100 gm. brain per minute, while with (*3*) it is between 7–11 ml. Physiologically inactive brains may also show a relatively high O_2 consumption with (*3*). It is suggested that by washing the blood corpuscles and by substituting a Ringer-albumin solution for plasma an inhibitor of brain metabolism is eliminated.

Venous pressure as a factor limiting tolerance of acceleration in the prone position. P. J. MAHER, JR. (by invitation), O. GAUER (by invitation), E. E. MARTIN (by invitation), J. P. HENRY. *Aero Medical Laby., Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio.* Practical considerations of flight control has stimulated the modification of the full prone position in order to permit better forward vision. The torso is raised until the head and chest make an angle of 25° with the horizontal. Standard light tests of visual symptoms and electrocardiograph recordings were carried out with 7 unrelaxed subjects in the range of 10–12 g. Visual symptoms were absent or mild. The electrocardiographic changes were of the same type and degree as those found during positive acceleration of approximately three g in the upright seated posture. However, 5 (5) subjects complained of intense pain in the legs or forearms during high accelerations. Petechiae were observed in 3 subjects, extending up the arms to the biceps region. Venous pressures were measured directly in 2 (2) subjects at 10 g. using a vein in the forearm and recording pressures by means of a trimount inductance gauge. Pressures of 175 mm. Hg were recorded in both cases. Since the vertical heart-leg distance was 50% greater than the heart-arm distance of approximately 19 cm., even larger pressures probably occur in the legs. These results suggest that pain in the extremities may determine the upper limit of g-tolerance in the prone position.

Repeated explosive decompressions. DAVID I. MAHONEY (introduced by J. W. WILSON). *Aero Medical Laboratory, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio.* As a result of increasing interest in the explosive decompression of pressurized aircraft, the question has arisen concerning the possibility of producing irreversible damage in individuals exposed to explosive decompressions of moderate intensity repeatedly carried out within a period of several hours. In order to test this possibility, a series of experiments was conducted using dogs as subjects. Thirteen test animals were used during the study and were subjected to no surgical procedure. The animals singly or in pairs, were placed in a small cabin which was located within a low-pressure chamber. The small chamber was held at ground level until the large chamber was evacuated to the altitude required to give the desired pressure differential (6.55 psi). A membrane of several layers of brown wrapping paper covering a $10\frac{1}{2}$ in. diameter orifice in the small chamber was broken at the desired time of decompression. The chamber was then immediately brought to ground level to preclude any effects of hypoxia. The time for all explosions varied between 0.120 to 0.126 seconds. The total time elapsing during a series of 7 explosive decompressions on each dog ranged from 45 to 75 minutes. There was no evidence of ill effects from the explosive decompressions in any of the dogs either immediately after the decompressions or during a subsequent observation period of 2 weeks. The lack of evidence of irreversible damage in this series plus the results of Hitchcock *et al.* (*J. Av. Med.* 17 : 6, 1946) lead to the conclusion that repeated explosive decompressions of moderate intensity are not a significant factor that may lead to mortality in dogs.

Behavior of actomyosin threads. SAMUEL MALLOV (by invitation) and JANE SANDS ROBB. *Dept. of Pharmacology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* Actomyosin from skeletal muscle of the rabbit, dog and guinea pig has been extracted and also purified by methods described by Szent Gyorgyi. Mincing favors greater output in shorter time but renders further separation more difficult. Purified actomyosin is stable for several weeks. Threads can be drawn but in contrast to unpurified threads, these respond to ATP only if calcium and/or magnesium ions are added and even then the degree of shortening is less. Images of these contracting threads have been projected to a screen and moving pictures taken.

Movement, generally beginning at the ends, is preceded by increase in density. As shortening proceeds, spiraling occurs. It is our impression that threads drawn into about 0.015% magnesium chloride develop tighter spirals (than is the case when drawn in potassium chloride) and that shortening is followed by partial relaxation. Behavior of threads drawn into 1:2,000,000 solution of crystalline cardiac glycoside is standard. If the glycoside is mixed with actomyosin and allowed to stand for an hour or two before threads are drawn considerable relaxation precedes contraction, spiraling is pronounced and maximum shortening appears. We have not been able to demonstrate activation by barium which was attempted because of the report of Deutsch and Lundin that it is barium and not ATP which is responsible for automatic cardiac activity. Using standard methods, less actomyosin can be extracted from a given amount of heart muscle; it is less viscid, forms threads less well, and these in spite of being thinner are less reactive than threads composed of skeletal actomyosin.

Intestinal Perfusion in a patient with chronic uremia. N. S. R. MALUF. *Surgical Research Unit, Brooke General Hospital, Fort Sam Houston, Texas.* Intestinal perfusion was tested in a severely uremic patient by the triple-bore tube. Thirty-one liters of 2 to 2.27% anhydrous sodium sulfate were dripped in 24 hours for intestinal and, then, gastric irrigation. The small quantity of liquid which regurgitated into the stomach while the intestine was being perfused was sucked through a gastric tube admitted through the other nostril; this completely prevented vomiting. Inevitable leakage through the anus was through a rubber-tube (1.6 cm. O.D.; 1.1 cm. I.D.) inserted into the rectum and attached to the buttocks by adhesive tape; this almost totally prevented soiling. Perfusion averaged 22.2 cc/min., which was much slower than previously used in non-uremic patients (Maluf, 1948, *Federation Proc.* 7: 77, 1948, *J. Urology* 60: 307, 1948). Because of the high plasma-urea and slow perfusion, the urea-clearance was only 1.8 cc/min. Nevertheless, 50 gm. of urea were extracted in 24 hours and plasma-urea fell from 357 to 246 mg/100 cc. in spite of intestinal perfusion occupying only 10.5 hours, the remaining 13 hours being exclusively gastric irrigation. Intestinal irrigation was at least 8 times more effective in eliminating urea than gastric. Edema, ascites and weight dropped markedly in spite of 6.5 liters liquid i.v. Elevated arterial pressure fell and the patient was mentally improved. 662 mg. of Ca, 2.3 gm. of K, and 48.3 gm. of Cl were lost in the perfusate. Plasma Cl (as NaCl) fell from 574.6 to 523.9 mg/100 cc. Besides dextrose, water and vitamins, 15 gm. NaCl and 4 gm. calcium gluconate were given intravenously.

Inactivation of thromboplastin by serum. F. D. MANN and MARGARET HURN (Introduced by T. B. MAGATH). *Division of Clinical Labys., Mayo Clinic, Rochester, Minn.* Most of the thromboplastic activity of extract of rabbit brain disappeared on incubation with an equal volume of human serum for 1 hour at 37°C. By dilution and acidification of the mixture, thromboplastin was precipitated and recovered in active form. Decalcification of the serum with oxalate largely prevented the inactivation of thromboplastin. Over the *pH* range 7 to 8, alkali decidedly favored and acid inhibited the inactivation of thromboplastin by serum. A rather similar *pH* effect was observed for conversion of prothrombin to thrombin in the two-stage system.

Effect of dibenamine on ventricular fibrillation following sudden coronary occlusion. G. W. MANNING. *R.C.A.F. Institute of Aviation Medicine, Toronto, and the Dept. of Physiology, Univ. of Western Ontario, Ontario, Canada.* In 75% of normal conscious dogs sudden occlusion of the left circumflex coronary artery results in ventricular tachycardia followed by ventricular fibrillation and death. It has been shown that cardiac sympathetic denervation and the sympatheticolytic drug, di hydro ergotamine, significantly inhibits the development of this fatal ectopic rhythm. In view of the results reported by Nickerson *et al.*, with dibenamine, the effect of this sympathetic blocking agent on the immediate mortality due to ventricular fibrillation was studied. Under anesthesia a loose ligature was placed around the left circumflex coronary artery. Dibenamine (20 mg/kg.) was given intravenously in 250 cc. of normal saline, over a period of ½ to ¾ of an hour. E.C.G. and blood pressures, in some cases, were recorded before and after dibenamine and before, during and after ligation. Sudden ligation in the conscious dog was carried out the following day by traction on the ends of the ligature which had been brought out through the chest wall (at a tangent to the heart.) In the E.C.G. the most constant changes noted were an increase in the heart rate, and increase in amplitude of the T and P waves and a reduction of the amplitude of the R waves occurred in some experiments following the injection of dibenamine. Following ligation of the left circumflex the usual sequence of events was noted in the E.C.G. There was a progressive elevation of the S-T segments, frequent extrasystoles and bouts of ventricular tachycardia followed by fatal ventricular fibrillation in 55% of the animals within the 24-hour period following ligation—(all occurred within the first 12 minutes

except in one animal which died in 10–12 hours following ligation). In the control series the same changes were observed and 75% of the animals died in ventricular fibrillation within the 24-hour period. Although an insignificant reduction in mortality occurred, the frequency of extrasystoles and ventricular tachycardia appeared to be no different from that seen in untreated control animals. It would appear from these experiments that dibenamine is not an effective agent in preventing the fatal ectopic cardiac rhythm which occurs following sudden coronary occlusion in the conscious dog.

Characteristics of the thigh muscles with a double action. J. E. MARKEE and MAUDE WILLIAMS (by invitation). *Dept. of Anatomy, Duke Univ. School of Medicine, Durham, N. C. and Division of Physiology, Womens College of Univ. North Carolina, Greensboro, N. C.* Previously, evidence was presented that muscles which pass over both the knee and hip function as double muscles; one part shortens to move the hip; another part shortens to move the thigh. This different participation of the separately innervated areas of the biceps femoris, semitendenosus, semimembranosus, rectus femoris, sartorius and gracilis occur during *1*) direct stimulation of the nerve branches; *2*) reflex flexion and crossed extension elicited in decerebrate preparations; and *3*) flexion and extension induced by cortical stimulation. In the present investigation, evidence is presented indicating that these thigh muscles which act as double muscles possess the functional characteristics of simple flexor and extensor muscles. That is, one part of the biceps femoris flexes the knee and resembles a simple flexor muscle in that it fatigues rapidly and develops less tension per unit of weight. In addition the development of effective tension occurs through a longer proportion of the range of shortening of the fibers. On the other hand, the part of the biceps femoris which extends the hip resembles muscles which are simple extensors in that this part fatigues less rapidly and develops more tension per unit of weight. In addition the development of tension is more dependent on the length of the fiber in the extensor portion of the muscle. The flexor and extensor portions of the semitendenosus and gracilis respectively possess these flexor and extensor characteristics.

Specific resistance of regenerating Dugesia in different media and the electrical work of polarity control. GORDON MARSH and JEAN DIMMITT (by invitation). *Zoological Labys., State Univ. of Iowa, Iowa City, Ia.* Pieces of *Dugesia tigrina*, regenerated 3 days in solutions of different specific resistance, were transferred individually to a chamber 0.149 mm. deep, which confined them at virtually uniform thickness. The voltage difference producing constant current with and without the piece, divided by that current, gives the difference in resistance, under the two conditions, of a chamber segment of length equal to the piece. During current flow the orientation, geometric shape and critical dimensions of the piece were determined, enabling calculation of the 'specific resistances' (ohm-cm. at 24°C.):

SP. RES. MEDIUM	SP. RES. DUGESIA	NO. CASES	Q_{O_2}
923	1682 ± 178	9	159–474
2280	1587 ± 203	10	140–400
5180	2370 ± 330	12	122–349
10,900	4538 ± 451	13	113–332

Midpieces showed the highest and posterior pieces the lowest resistance, with significance doubtful. Resistance was independent of current strength, which was usually that capable of reversing polarity. The previously demonstrated constancy of potential gradient producing a given morphogenetic effect in media of varying resistance, gains significance from the fact that the internal current varies. The electrical energy expended in a 1 $mm.^3$ piece regenerating 4 days in the current varies from 0.06 to 0.25 cal., depending upon specific resistance and potential gradient (regressive bipolarity 1.78 volts/cm.; reversal 2.17 volts/cm.). Assuming 1.05 for piece density and 5 cal/cc. caloric equivalent for oxygen, these energies would represent the Q_{O_2}'s above if provided by the regenerating material. All lie within the limits of published data.

Purification and possible histochemical localization of renin. JOHN MARSHALL (by invitation) and G. E. WAKERLIN. *Depts. of Physiology and Pathology, Univ. of Illinois College of Medicine, Chicago, Ill.* Purified hog renin was prepared by an extension of the ethanol fractionation method previously described (*Federation Proc.* 7: 78, 1948), with a resulting yield of 22,000 DU (Goldblatt) at a purity of 125 DU/mg.N. This preparation gave a polysaccharide reaction to the Hotchkiss technique (*Arch. Biochem.* 16: 131, 1948). Accordingly, sections of 22 dog kidneys of widely varying renin content were stained by the Hotchkiss method. Renin content varied from 0 to 22 DU/gm. The results showed a good correlation between the renin content and granules containing glycoprotein in the juxtaglomerular apparatus. The solubility of the glycoprotein granules in the juxtaglomerular apparatus of tissues prepared by freezing and drying was compared with that of dog renin. Using the same procedures employed for the fractional separation of purified renin, it was found that the solubilities of both substances mentioned above corresponded. Both were insoluble at 0°C., ionic strength 0.005, *p*H 5.10, at 20% ethanol con-

centrations or higher, and were soluble at lower ethanol concentrations.

Presence of multiple cortical foci in the primary sensory projection systems of the cat. WADE H. MARSHALL. *National Institutes of Health, Bethesda Md.* In connection with work on the recovery cycle of the somatic sensory system of the cat, this author located 3 cortical regions at which responses occurred following tactile stimulation of the contralateral forefoot. These regions were specified in a joint paper on cortical reactions (Marshall *et. al., J. Neurophysiol.* 4: 1, 1941). Adrian (*J. Physiol.* 100: 159, 1941) has questioned the presence of 3 such regions. Woolsey, in his published work on mapping the somatic sensory cortex of the cat, has discussed only 2 of these regions. One, the sensory region of Campbell, he has logically labeled *Somatic I*; the other, the anterior ectosylvian region, he has labeled *Somatic II*. The question of the third he does not discuss. The point has been reexamined and the author's original findings have been confirmed. There is a third area extending laterally from the region of the junction of ansate and lateral sulci across the posterior part of the *Somatic I* face area toward the anterior ectosylvian *Somatic II*. Multiple primary projection foci are not peculiar to the somatic sensory system. The visual system in the cat contains 2 demonstrable areas in addition to the striate and contiguous boundary region. One of these is in the posterior suprasylvian gyrus. The other region is in the middle ectosylvian gyrus extending medially across the suprasylvian sulcus onto the dorsal surface of the suprasylvian gyrus.

Suppressor action on primary sensory projection reactions. WADE H. MARSHALL. *National Institutes of Health, Bethesda, Md.* Previous work by the author has shown significant bilateral interfiber interaction in optic tract and radiations of the cat. It was considered desirable to determine if cortical suppression (suppressor reaction of Leão, and Leão and Morrison) would exercise a suppressor action on the radiation terminations. A decision on this question necessarily involves simultaneous observations on the primary relay nuclei of the thalamus. This has been done in the geniculate-striate system of the rabbit and cat. In the rabbit no change has been observed in geniculate reactions or in the radiation spike complex as recorded on, in or under the cortex. The decision in the cat is not yet clear because of the difficulty in eliciting regular and repeated suppressor cycles in that animal. The radiation amplitude has shown reduction in some experiments accompanied by a reduction in lateral geniculate postsynaptic reaction. However, it is certain, in the case of the cat, that radiation amplitude is reduced not more than 10%, if any such reduction occurs due to the suppressor reaction. In both cat and rabbit the suppressor reaction reduces and eliminates the primary surface positive cortical components. The first cortical synaptic reaction is the last to be completely eliminated. In the rabbit it is certain that the reaction affects only the purely cortical events. Subcortical or intracortical block of afferent impulses does not occur; only the primary cortical events subsequent to radiation activity are affected.

Static assessment of the relative efficiency of anti-G suits. E. E. MARTIN (by invitation), G. SCHROER (by invitation), J. P. HENRY. *Aero Medical Laby., Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio.* Attempts to estimate the effectiveness of anti-G suits while at rest by measuring the changes they induce in blood pressure have shown increments proportionate to the relative efficiency of these devices during acceleration. (Air Materiel Command Memorandum Report MCREXD-689-2C, dated July 1948.) The results suggest that tests of G-suit efficiency could be devised which do not require the use of a human centrifuge. With this end in view, 3 further measurements were made: venous pressure, vital capacity and teeter board weight shift. Venous pressure was measured at the antecubital fossa when the legs only of the G-3A, G-4A, and G-5 suits were inflated. A teeter board was employed to measure the weight shift induced by the inflation. The changes in vital capacity were measured under the same conditions. Eight series of 3 tests each were carried out on 6 subjects. The following table shows the changes induced by suit inflation:

	G-3A	G-4A	G-5
Venous pressure increase (cm. H_2O)	0.3 ± 0.2	1.9 ± 0.6	4.3 ± 0.5
Vital capacity decrease (cc.)	250 ± 60	325 ± 70	400 ± 60
Teeter Board weight shift (pounds)	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.3

These differences are qualitatively confirmed by the results of tests of these suits on the human centrifuge. In the G-3A suit mean protection as estimated from blood pressure changes was 1.0G, G-4A suit 2.2G, and the G-5 suit 2.3G.

Occurrence of potassium U/P ratios lower than 1.0. F. MATEER (by invitation), L. GREENMAN (by invitation), J. H. PETERS (by invitation), R. C. GOW (by invitation), and T. S. DANOWSKI. *Dept. of Research Medicine, Univ. of Pittsburgh School of Medicine, Pittsburgh, Penna.* It has been demonstrated that during periods of reduced or absent intake of food and water the urinary excretion of sodium decreases to zero while potassium continues to be present in the urine (*J. Clin. Invest.* 23:

807, 1944). This diminished excretion of sodium has been interpreted as a salt-conserving response preventing sodium depletion. The continued urinary loss of potassium is often associated with transfers of potassium out of cells, in part with the negative balance of portein and in part with dehydration (*J. Clin. Invest.* 23: 93, 1944). These transfers may not occur during renal suppression (*J. Clin. Invest.* 27: 74, 1948). If urine is elaborated, excretion of potassium continues, thereby permitting movement of cellular potassium without production of toxic extracellular levels. This urinary loss of potassium frequently continues even in the presence of hypopotassemia and is one of the important routes of loss resulting in potassium depletion (*J. Clin. Invest.* 27: 557, 1948). This would suggest that the kidney is incapable of conserving potassium when body stores of this cation are depleted. Our studies of subjects with hypokalemia indicate, however, that the urinary loss of potassium may sometimes be markedly reduced, resulting in potassium U/P ratios lower than 1.0. In the 6 instances in which this was observed, 3 patients recovering from diabetic acidosis and 3 infants with protracted vomiting, the decrease in urine potassium concentrations below those in serum did not necessarily appear coincidental with hypopotassemia but rather became evident after several days of persistent deficits of potassium.

Measurement of renal venous pressure in normal man and in patients with congestive heart failure. M. Harrison Maxwell, Ernest S. Breed and Irving L. Schwartz (introduced by Homer W. Smith). *Dept. of Physiology, New York Univ. College of Medicine, New York City.* Direct measurements of renal venous pressure were recorded in 7 normal male subjects and in 5 male patients in severe chronic congestive heart failure by means of a no. 10 Cournand radio-opaque catheter passed under fluoroscopy from an antecubital vein into the right renal vein. A saline manometer was used with repeated elevation above equilibrium. The normal subjects were all supine, the cardiacs supine or semi-recumbent. An arbitrary zero point of 10 cm. above the table top was assumed in all cases. Lateral X-rays taken on 6 subjects showed the tip of the catheter *in situ* varied from 5.3 to 15.2 cm. above the table top, with an average of 9.9. Since the true zero point coincided so closely with the assumed one, no corrections were necessary. The renal venous pressure in normal subjects ranged from 13.0 to 19.8 cm. of saline, with an average of 15.4; in failure the pressure ranged from 16.5 to 28.0 cm., with an average of 24.3. In 6 normal patients and 3 cardiac patients, right atrial pressures were recorded during withdrawal of the catheter, the zero point being taken as 5 cm. below the angle of Louis. The normal subjects had pressures varying from 0 to 5.3 cm. of saline, with an average of 2.5. The cardiac subjects had pressures varying from 11.0 to 15.0 cm., with an average of 13.0.

Determination of blood flow through the finger at high rates of flow. Jere Mead and Robert C. Schoenfeld (introduced by H. S. Belding). *The Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass.* When venous occlusion is accomplished in the finger without occlusion artefact, the slope of the tracing at the instant following occlusion depicts a volume rate of change which is the algebraic sum of two components: *1*) the volume rate of change varying through each cardiac cycle to produce the finger pulse; *2*) the volume rate of change which relates directly to the obstruction of outflow from the finger. If the instantaneous pulse slope derived from corresponding points on preceding pulse waves is subtracted algebraically from the initial occlusion slope, the volume rate of change of the finger independent of the pulse volume rate of change is obtained. This represents the instantaneous rate of blood flow through the finger.

Utilizing an electric solenoid air valve 'triggered' through a variable time delay circuit by the QRS potential of the ECG, venous occlusions were produced at specific points through the cardiac cycle, and variations in blood flow during the finger pulse cycle were studied. In vasodilated individuals the rate of flow in the terminal phalanx of the finger increased during the rising phase of the pulse to maximum values as high as 200 cc/100 cc. of tissue/minute, and decreased during the diminishing phase of the pulse to roughly ⅔ of the maximum flow. Average flows over the pulse cycle as high as 160 cc/100 cc. of tissue/minute were computed. The markedly pulsatile character of blood flow through the terminal phalanx of the finger suggests that a considerable part of the finger's blood flow may be through the arteriovenous shunts described in this region.

Effect of carbohydrate on starvation metabolism in the presence of a high caloric deficit. G. W. Mellinger (introduced by J. W. Heim). *Aero Medical Laboratory, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio.* During a study of airborne survival ratios, metabolic observations were made to determine the effect of feeding 100 gm. of carbohydrate daily on the body protein loss, ketosis, water balance and physical efficiency of men performing strenuous physical exertion in a cold environment. Two subjects resided continuously in an all-weather chamber at −29°C. for 6 days. During this period they expended 4000 to 4500 calories while ingesting only 100 gm. of carbohydrate (400 cal.) daily. Clothing consisted of an experimental arctic clothing assembly having a clo value of approximately four. Sleeping bags were used at night. As a control, the subjects performed a similar

amount of work for 6 days while fasting. The serum carbon dioxide, organic acids, urinary ammonia and titratible acidity showed that there was a moderate degree of ketosis during the fasting experiments, but that all but slight changes were prevented by carbohydrate in amounts of 100 gm daily. Body protein loss was spared to the extent of 40–50% of the fasting loss when the carbohydrate was given. The water balance was favorably affected by 100 gm. of carbohydrate as was shown by the marked reduction in the minimum urine water and total urine solutes, and by the moderate reduction in the minimum water intake. While maximum efficiency was not maintained during the experiments, the subjects were in sufficiently good condition at the end of the 6 days to perform tasks necessary for survival.

Further studies on complete parenteral alimentation. H. C. MENG (introduced by HOWARD J. CURTIS). *Dept. of Physiology, Vanderbilt Univ. Medical School, Nashville, Tenn.* A fat emulsion has been prepared for intravenous feeding (described elsewhere), and the present report describes the nutritional value and toxicity of this emulsion in dogs and rabbits. In the control period, 5 adult dogs were fed a complete basal diet by mouth. Immediately following this they were fed entirely by vein for 4 weeks and then killed for pathological examination. Four were given the same diet as during the control period, of which 34% of the calories were from fat, while the 5th dog received the same carbohydrate and protein diet but without the fat. The dogs on the complete diet were normal and healthy in all respects, and all laboratory findings were negative. The dog on the fat-free diet lost 14% of his weight and was very weak and apathetic. The injection of relatively large amounts of the emulsion in rabbits produced no appreciable pyrogenic effects. Further, the daily injection in rabbits of large quantities of both the emulsion and the emulsifying agents for as long as 4 weeks produced no deleterious effects. As tested in rabbits, it has been found that the emulsion is not antigenic. It is concluded that this emulsion is non-toxic and that the body is capable of utilizing fat in this form.

Physiologic effects of bilateral simultaneous removal of Brodmann cytoarchitectural areas in the human. FRED A. METTLER. *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.* In the 'combined-operations' study, known as the Columbia-Greystone Project, the physiology of 23 psychotic individuals from whom localized areas of frontal cortex were simultaneously, bilaterally removed, was studied pre- and postoperatively. Data were collected relating to formed elements of the blood, blood chemistry, blood pressure, body weight, gastrointestinal motility, electroencephalography, vision audition, somatosensation, reflex state, motor function and higher cerebral functions. The studies were begun 40 days prior to operation and have been continued, when pertinent, for over 1½ years. In certain physiologic fields (basal metabolism, somatic activity) no data could be obtained.

Bilateral removal of Brodmann areas of granular frontal cortex or of various combinations of these produced no definite, enduring, physiologic changes in any of the fields noted above except, in some cases, electroencephalography. Temporary effects were short-lived and generally not of such a nature as to demonstrate a causal effect between the nature of the ablation and the physiologic disturbance. Aphasia does not follow removal of *area 44*. The effects of removal of agranular cortex were also relatively unproductive. As previously reported for the simian (by Hines *et al.* and by the present author) cortex of *area 6* may be removed without evoking evidence of spasticity. Neither does such a procedure necessarily produce forced grasp in the human.

III. Effect of low barometric pressure on the brains of five vertebrates. BERNARD METZ (introduced by CHARLES G. WILBER). *Biological Laby., Fordham Univ., New York City.* Five vertebrates (the goldfish, *Carassius auratus*; the frog, *Rana pipiens*; the painted turtle, *Chrysemys picta*; the pigeon, *Columba livia*; and the Wistar rat, *Rattus norvegicus*) were subjected to simulated altitudes of 18,000, 33,000, 48,000, 64,000 and 90,000 feet, respectively. The survival rates, behavior and effects upon the vascular tissue and nervous elements of the brain were studied. The methods used and the survival rates were previously reported by Metz and Wilber (*Anat. Rec.* 99: 676, 1947; 101: 738, 1948; in the latter paper, survival rates for rats at 48,000 feet should be amended to read 66.2 seconds, not 66.2 minutes). Concerning the effects upon the brain, the most striking pathological changes are the intracranial hemorrhages (table 1). Visible damage to the nervous elements was noted only in a few cases. This nerve cell damage, as in the case of the hemorrhages, was widely dispersed in the optic lobes (in the fish, frog and turtle), cerebrum, cerebellum and medulla oblongata.

Table 1. Numbers of cases showing pathological changes

ANIMAL	NO. OF EXPERIMENTAL ANIMALS	HEMORRHAGES	NERVE CELL DAMAGE
Goldfish	50	36	5
Frog	50	23	3
Turtle	50	25	9
Pigeon	50	24	4
Rat	50	27	8

The fact that there was only a small number of cases showing nerve cell damage does not imply that the nervous elements of the brains of the other experimental animals escaped unharmed. There exists the possibility that the changes which may have occurred are not morphological in nature, but rather are biochemical phenomena at a submicroscopic level (Page. *Chemistry of the Brain*, 1937).

Renal hemodynamics during short and long periods of salt poor albumin administration. ALEXANDER J. MICHIE (by invitation), NICHOLAS S. GIMBEL (by invitation) and CECILIA RIEGEL. *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* The daily intravenous administration of salt-poor albumin to normal subjects caused a progressive expansion in plasma volume, an enlargement in extracellular fluid volume, an increasing venous pressure, a dilation of the roentgen heart shadow and an engorgement of the liver. The distended plasma volume produced a renal hyperemia and relaxation of efferent arteriolar tone. In renal hyperemia absence of a maximal filtration rate might be indicative of some systemic circulatory derangement. One week after termination of the albumin therapy and in the presence of a continued renal hyperemia the filtration rate increased as plasma volume and venous pressure dropped. Five months later the same subject was given 75.0 gm. of salt-poor albumin intravenously in 20 minutes. Renal plasma flow was raised 700 cc/min., and filtration rate showed a maximal increase of 40.0 cc/min. above the control values. The filtration fraction was decreased to a low of 0.117. The protracted renal hyperemia apparently led to an increase in Tm_{PAH}. As the albumin was slowly metabolized during the 2- to 3-week experimental period, these subjects approached a protein-free diet, which in the experimental animal tends to diminish filtration rate, renal plasma flow and tubular mass. In the acute experiment 75.0 gm. of albumin caused no significant change in Tm_{PAH} or Tm_G. The unchanged Tm_G shows that no aglomerular arteriovenous shunts were opened by this hyperemia and that the diminution in the PAH extraction ratio is probably due to increased albumin binding.

Influence of adrenaline on the cardiodepressor effect of acetylcholine and the vagus nerve. S. MIDDLETON and J. TALESNIK (introduced by HAROLD C. WIGGERS). *Physiological Institute, Univ. of Chile, Santiago, Chile.* In isolated guinea pig and cat hearts adrenaline perfusion with concentrations of 3–20 μg/l. produced a marked increase of submaximal cardiodepressor (negative chrono and inotropic) effects of acetylcholine (0.05–0.5 μg.). This augmenting effect was less marked with concentrations of adrenaline of 30 μg/l. or more, while concentrations of 1 μg/l. or less were ineffective. The effects of adrenaline were not influenced when nicotine in concentrations of 1-2 μg/l. was added to the perfusion fluid. This would indicate that adrenaline acts by modifying the sensitivity of the cardiac effectors (peripheral effect); an influence on the intracardiac ganglia should be discarded. Adrenaline did not influence the cardiodepressor action of KCl.

In 28 experiments performed on isolated cat hearts, perfusion with adrenaline in concentrations of 0.05–0.1 μg/l. produced a very marked increase of submaximal cardiodepressor effects induced by electrical excitation of the vagus nerves. Smaller concentrations of adrenaline were ineffective. Concentrations of 1 μg/l. or more produced a decrease of the vagal effects. In view of the above-described results with nicotine the effect of high concentrations of adrenaline are probably due to a blocking action on the intracardiac ganglia. Eight experiments were performed on frogs, in which perfusion of the isolated heart (Straub's heart with Krayer cannula) with adrenaline (0.5–1 μg/l.) produced a marked increase of the cardiodepressor effect of the vagus.

Influence of the cardiac sympathetic nerves on the cardiodepressor action of the vagus nerve. S. MIDDLETON and J. TALESNIK (introduced by HAROLD C. WIGGERS). *Physiological Institute, Univ. of Chile, Santiago, Chile.* Twenty-five experiments were performed on isolated cat hearts, which were removed with the vagi and part of the surrounding mediastinal structures (including the stellate ganglia and adjacent thoracic sympathetic chains) attached. The preparations were made in such a way that, when desired, the stellate ganglion could also be perfused. In various experiments the vagi or the preganglionic or postganglionic sympathetic nerves were electrically stimulated. It was observed that stimulation of the cardiac pre- or postganglionic sympathetics in 6 experiments induced increase of submaximal cardiodepressor effect of the vagus. In 8 experiments, however, the sympathetic produced depression of the vagal effects and in 11 experiments the latter were not significantly modified. Nine experiments were conducted on intact cats. Much more regular results were obtained in this series, compared with the isolated heart. In all the animals, stimulation of the sympathetic cardiac nerves which did not by themselves produce changes in cardiac rate (subthreshold), induced, however, marked increases of submaximal bradycardic effects of the vagus. Similar results were obtained with threshold stimulation of the sympathetic. It seems interesting to point out that in the intact cat the cardiac response to stimulation of the sympathetic nerves is very minimal and rare. Usually only negligible effects were observed even with supramaximal stimulation. In the isolated heart, on the contrary,

the effects of the sympathetic nerves on cardiac activity are very manifest.

Effect of pH on the three waves of the protein polarogram. *G. J. Millar* (introduced by L. B. JAQUES). *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada.* Protein added to an ammonia buffer containing cobalt yields a polarogram exhibiting 3 waves (Jurka, *Collection Czech. Chem. Commun.* 11: 216, 1939; Millar, *Proc. Can. Physiol. Soc.* 1947). When the *p*H of the medium is altered, the magnitudes of the first two waves are either unaffected or are decreased as the *p*H falls, while that of the third ('prenatrium') wave is enhanced. The following modification of Brdicka's reactions is proposed to account for the behaviour of the second wave:—SH + e $\rightarrow$ $-S^-$ + H; $-S^-$ + $H_3O^+ \rightleftarrows$ —SH + H_2O. The observed *p*H effect seems to depend on the concentration of cobalt—NH_3 complex. The cobaltous complex dissociates easily as the *p*H is lowered. In the presence of the stable cobaltic complex, the *p*H of the medium may be lowered to 3.5 with little effect on the height of the first two waves. Hence, these waves are only indirectly dependent on *p*H.

Experiments with acetylated proteins and some organic compounds suggest that the third wave depends on the free amino groups of the protein and possibly on histidine residues. The following reactions are proposed: $-NH_3^+ + e \rightarrow -NH_2 + H$; $-NH_2$ + $H_3O^+ \rightleftarrows$ $-NH_3^+$ + H_2O. When the *p*H is lowered a greater proportion of free amino groups are in the zwitterion form and the third wave is enhanced to a maximal value. Its height is probably related to the protein dissociation curve.

Effect of bacitracin on renal function. JOHN H. MILLER (by invitation), ROGER K. MCDONALD (by invitation) and NATHAN W. SHOCK. *Section on Cardiovascular Diseases and Gerontology, National Heart Institute, National Institutes of Health, Bethesda, Md., and the Baltimore City Hospitals, Baltimore, Md.* The parenteral administration of bacitracin is productive of proteinuria in patients treated for various infections. In the present study, serial renal function tests were performed on 12 subjects, free of infection, who received intramuscular injections of 1500 U of bacitracin per kilogram body weight on two successive days. Proteinuria appeared in all individuals at 48–60 hours after the first injection, was maximal during the first 24–48 hours after its appearance (range of maximal 24-hour proteinuria was 0.7–9.7 gm.), and persisted for an average of 10 days. Microscopic examination of the urine revealed numerous tubular epithelial cells and an absence of red cells at the time of maximal proteinuria. Tm_{PAH} was decreased at the time of maximal proteinuria from 15–85% (mean 39%). Tm_G was decreased from 20–76% (mean 52%). The maximal tubular excretory capacity for PAH and the maximal tubular reabsorptive capacity for glucose returned gradually over a period of 3 to 8 weeks to 88% or more of the baseline value in 10 to 12 subjects. In the other 2 subjects, Tm_{PAH} was still depressed 25% at 8 weeks. Inulin clearance was decreased from 2–75% (mean 32%), and the PAH clearance was depressed from 0–76% (mean 24%) during maximal proteinuria. Both PAH and inulin clearances tended to return to baseline levels more rapidly than did the Tm values. Unless the nephrotoxic principle contained in present preparations of bacitracin is an impurity which can be eliminated, these findings preclude the general use of this antibiotic by parenteral administration.

Effects of growth hormone on the blood sugar and insulin sensitivity of the rat. ANNE E. MILMAN (by invitation) and JANE A. RUSSELL. *Dept. of Physiological Chemistry, Yale Univ., New Haven, Conn.* Observations were made concerning the effects of a highly purified preparation of growth hormone (prepared by Wilhelmi, Fishman, and Russell) on the blood sugar and on the insulin sensitivity of fasted normal and diabetic rats. When doses of 3 to 5 mg/100 gm. were injected intraperitoneally into mature, normal or adrenalectomized male animals, a significant and prolonged depression of the blood sugar followed. In alloxanized or partially depancreatized rats, on the other hand, these same preparations of growth hormone produced a marked rise in blood sugar. This occurred in animals which were only mildly diabetic as well as those with more severe symptoms. Normal animals pretreated with growth hormone 4 to 6 hours before were less sensitive to insulin. Similarly, in diabetic animals, the duration of the blood sugar lowering effect of subcutaneously administered protamine-zinc-insulin was curtailed by injections of growth hormone (given 2–3 hours after the insulin). Growth hormone did not appear to alter the glucose tolerance of normal animals under our experimental conditions. Bioassay of some of the growth hormone preparations showed traces of adrenotrophic hormone to be present; these amounts of ACTH were found to be to be insufficient to reproduce the effects observed in diabetic animals. These results suggest that in the normal rat, the administration of growth hormone may provoke secretion of extra insulin. In the animal lacking sufficient islet tissue, hyperglycemic or contra-insulin effects of the growth hormone are seen instead.

Influence of intestinal motility on intestinal blood flow. M. SIDKY MOHAMED (by invitation) and JOHN W. BEAN. *Dept. of Physiology, Univ. of Michigan, Ann Arbor, Mich.* The artery and vein to a loop of intestine of dogs anesthetized with pentothal were cannulated and the isolated loop set up in a perfusion apparatus. Oxygenated heparinized dog blood was supplied to the tissue

under constant or pulsatile pressure. The venous outflow of blood was continuously recorded and the motility of the loop recorded from the lumen by tambour. On initiating the perfusion of quiescent gut the venous outflow was continuous but with the return of rhythmical contractions it became intermittent, increasing sharply during the contraction phase, decreasing or even momentarily stopping with relaxation. Since flow speeded up during the contraction phase the intermittence cannot be due simply to a cutting off of the arterial supply by occlusion of the vessels during the contraction, thus indicating the gut contraction causes an active propulsion of the blood from the tissue. Interruption of rhythmic contractions by periods of relative quiescence, at which time the arterial pressure should be most effective in increasing the flow, resulted in a decreased flow. The flow was also markedly augmented during the contraction phase of the longer tonic waves upon which the rhythmic contractions are superimposed but excessively strong tonic contractions, after initially increasing the flow, stopped the flow completely during period of high tonus presumably by passive vascular occlusion. On experimental occlusion of the venous outflow, venous pressure increased progressively and intermittently, coincident with successive rhythmical contraction phases. The experiments demonstrate the importance of intestinal motility in blood flow through the gut. Similar effects were with pulsatile and constant perfusion pressure.

Studies on vasotropic principles in blood (VEM and VDM) and renal hemodynamics in chronic heart failure. R. Mokotoff, D. J. W. Escher, I. S. Edelman, J. Grossman and L. Leiter (all by invitation) and R. E. Weston, B. W. Zweifach and E. Shorr. *Medical Division, Montefiore Hospital, and the Dept. of Medicine, Cornell Univ. Medical College, the New York Hospital, New York City.* Since the increased renal arteriolar constriction in chronic congestive heart failure is probably not neurogenic, the possible role of VEM and VDM, newly described vasotropic principles, was investigated. Blood samples from the renal and hepatic veins, and the femoral artery or normal controls and of patients in chronic failure, were assayed for VEM and VDM by the rat mesoappendix test, in which the potentiation or inhibition of the arteriolar responsiveness to epinephrine is measured. Simultaneous renal hemodynamic and renal and hepatic oxygen extraction studies were performed. In all cases of congestive failure were *both* an increased filtration fraction and an increased oxygen extraction ratio were demonstrated, there were significant amounts of vasoexcitor principle (VEM) in the renal vein blood. In contrast, with one exception, almost no VEM was detected in the controls. The cardiacs had markedly reduced glomerular filtration rates and renal blood flows with renal oxygen extraction increased to 14–29% as compared to 5–9% in the controls. Pronounced vasodepressor activity (VDM) was found in all hepatic vein samples of congestive failure patients. Only one control showed mild VDM. Although femoral arterial blood containing a mixture of VEM and VDM showed a slight predominance of the vasoexcitor principle in the majority of cardiacs, several did show a peripheral blood VDM predominance. This is important in view of the recent demonstration by Baez, Mazur and Shorr, that VDM has a profound antidiuretic effect. The relation of these principles to impaired renal hemodynamics in congestive failure will be discussed.

Method for the determination of heparin in blood. F. C. Monkhouse (by invitation), Mary Stewart (by invitation) and L. B. Jaques. *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada.* A method for the determination of heparin in blood has been developed. It is applicable to the estimation of raised quantities of heparin in blood, as after intravenous injection, or anaphylactic shock. Blood is received into citrate and the heparin precipitated from the plasma with 7% octylamine hydrochloride. The precipitate is hydrolysed with 0.1N NaOH and the octylamine removed with ether. The heparin is then precipitated with 5% brucine phosphate at *p*H 6.0, and the brucine-heparin is washed with alcohol and ether and dried. The heparin present is assayed for its metachromatic activity with Azure-A using the method of Jaques, Mitford and Ricker (*Rev. Can. Biol.* 6: 740, 1947) or for antithrombin activity. Using the metachromatic method of assay, recoveries of 80–90% were obtained for heparin added to blood in concentrations of 0.2 to 3.6 mg/100 ml., while complete recovery of heparin was obtained from plasma. Normal human blood without added heparin gave a value equivalent to 0.009 mg. % of heparin by this method. Owing to the extremely small amounts of heparin thus found in normal blood, certain modifications of the method are indicated in applying it to the estimation of normal blood heparin.

Further studies on the stimulating effects of light on sexual activity of the female opossum during the nonbreeding season. Charles F. Morgan. *Dept. of Physiology and Biophysics, Georgetown Univ. School of Medicine, Washington, D. C.* Opossums were subjected to radiant energy from incandescent lamps, some with and some without monochromatic filters. The filters used were 'black' infra-red (λ8500), red (λ6400) and green (λ5460). The radiant energy was determined by means of a chromel-constantan thermopile connected to a sensitive Rubicon galvanometer. Increase in the size and weight of the reproductive tract appeared directly proportional to the amount

of radiant energy received through lamps with and without filters. Thus radiant energy changes show greater effect than changes in wave lengths.

One group of opossums was exposed to a total amount of unfiltered radiant energy given 10 hours per day and compared with another group of opossums given the same total amount of radiant energy extending 20 hours per day with both groups receiving the treatment the same number of days. The sizes and weights of the reproductive tracts in both groups were similar. The degree of stimulation was the same as above when the animals received an equal amount of radiant energy from flashing lights given over a 20-hour daily period. Direct observations of the ovaries at laparotomy revealed ovulations in some animals receiving the stimulating effects of infra-red radiations. Ovulations have not been obtained in animals injected with varying amounts of gonadotropic hormones.

Sudden increases of radiant energy to a greater constant daily amount resulted in stimulation as great as that obtained in experiments previously reported (Morgan, Chas. F. *Federation Proc.* 6: 359, 1947) in which incandescent light was increased by small increments each day such as occurs in nature.

Effect of spinal cord transection on the ventilatory response to exercise. DONALD P. MORGAN (by invitation) and FRED S. GRODINS. *Dept. of Physiology, Northwestern Univ. Medical School, Chicago, Ill.* The ventilatory response to electrically induced exercise of the hind limbs in barbitalized dogs was studied before and after section of the spinal cord at T_{10}. Respiratory rate, minute volume and oxygen consumption were determined at rest and during a steady state of exercise. Percutaneous electrical stimulation with 60-cycle AC modulated at 100/min. was used to produce oxygen consumptions up to seven times the resting value. A linear relationship was found between ventilation and O_2 consumption in 56 tests on 11 intact dogs The regression equation was: $Y = 0.56 + 0.031X$, where Y is ventilation in 1/min., BPTS, and X is oxygen consumption in cc/min., STPD. The correlation coefficient was 0.97. A linear relationship was also found in 50 tests on 11 dogs after cord section. The regression equation was: $Y = 2.31 + 0.027X$ and r was 0.78. Covariance analysis showed no significant difference between the regression coefficients, but a significantly higher adjusted mean ventilation in the sectioned animals. The latter was most apparent in the considerably higher resting ventilation after section. Respiratory patterns were different in the two groups, the tidal volume being less and the rate correspondingly greater at a given total ventilation in the sectioned animals.

Activity of single units in the cerebellar cortex. G. MORUZZI (by invitation), JOHN M. BROOKHART and R. S. SNIDER. *From the Depts. of Anatomy and Physiology, Northwestern Univ. Medical School, Chicago, Ill.* Spike potentials have been recorded from single units having somas located in the cerebellar cortex. Glass micropipette electrodes having tip diameters of 5 to 20 microns have been used for unipolar recordings in unanesthetized decerebrate cats. The recorded spikes have the following characteristics: *1*) duration of approximately 1 msec.; *2*) amplitudes reaching 250 microvolts, about 3 times the amplitude of the largest spontaneous cerebellar waves; *3*) unbroken contour with rising phase shorter than falling phase. The unitary origin is indicated by *1*) constancy of amplitude and contour, *2*) possibility of identifying multiple units in the same record and *3*) parallel modulation of amplitude of two units by slight cerebellar movements. Modification of spike frequency by local application of strychnine gives evidence of cortical localization of soma.

Spike activity falls into three classes: *1*) injury; *2*) normal spontaneous; and *3*) convulsive. Injury discharges are characterized by normal spike contour, by frequencies higher than 100/sec. and by durations usually less than one minute. Normal spontaneous spikes have been recorded at frequencies from 25 to 150/sec. for many minutes; they usually occur in outbursts of variable duration. Occasionally, spike discharges have been evoked or inhibited by sensory stimulation. Convulsive outbursts of spikes have been produced by local strychninization of the cerebellar cortex. After an initial increase in frequency, resulting from strychnine, characteristic outbursts of convulsive activity occur, starting at frequencies above 200/sec., increasing to terminal frequencies up to 500/sec., and terminating abruptly.

Influence of bulbo-reticular stimulation upon electrical activity of cerebral cortex. G. MORUZZI (by invitation) and H. W. MAGOUN. *Dept. of Anatomy, Northwestern Univ. Medical School, Chicago, Ill.* The effect of stimulating the suppressor bulbar reticular formation upon cortical electrical activity has been investigated in cats under chlorolosane anesthesia. The high voltage, 5/sec. waves, characteristically pronounced in the sensori-motor area with this anesthesia, were abolished during such bulbar stimulation; while lower voltage, faster, background activity continued. This effect was most complete in the ipsilateral sensori-motor cortex, but was observed also in the visual area and contralaterally. It was prevented by section of the midbrain tegmentum, but not by section of the tectum or both bases pedunculi. It remained after complete paralysis with curare, and was not reproduced by antidromic stimulation of the bulbar pyramid. This effect was obtained with stimuli to the reticular formation of 300/sec. and 2–3 V, with individual shocks of 1

msec. duration, i.e., with the same parameters of stimulation which suppressed spinal reflexes. With lower frequencies, higher voltages or longer shocks were required.

Effect of nephrotoxic sera on the clearance of urea, creatinine and para-aminohippurate in dogs. CAMPBELL MOSES and ELIZABETH R. THORNTON (by invitation). *Addison H. Gibson Laby. of the Univ. of Pittsburgh School of Medicine, Pittsburgh, Penna.* Nephrotoxic sera were produced in rabbits by the intraperitoneal injection of minced dog kidney after the method of Smadel (*J. Esptl. Med.* 64: 921, 1936). Considerable difficulty was encountered in producing a nephrotoxic serum that did not produce undesirable effects such as intractable vomiting and diarrhea. Before and at intervals after the administration of the serum urea clearance, creatinine clearance and para-aminohippurate clearance both for estimating renal blood flow and tubular excretory function were determined. Renal biopsies were made before and once or twice after the administration of the serum. In the 6 dogs in which this experiment was satisfactorily completed there was a lag of 6 to 12 days following the injection of the nephrotoxic sera before the signs of renal damage became evident. The administration of the sera produced a consistent decrease in urea clearance and creatinine clearance with only minor variations in renal blood flow. There was complete loss of tubular ability to excrete para-aminohippurate and in several instances the tubular excretion PAH fell below the glomerular filtration rate. This evidence together with the signs of only moderate glomerular and tubular damage in the biopsy specimens emphasizes the difficulty of accurately interpreting the quantitative significance of clearance in dogs in the presence of renal damage produced by nephrotoxic sera.

Rapid production of experimental atherosclerosis by producing kidney damage in the presence of hypercholesterolemia. CAMPBELL MOSES, ELIZABETH R. THORNTON (by invitation) and GRACE M. LONGABAUGH (by invitation). *Addison H. Gibson Laby. of the School of Medicine of the Univ. of Pittsburgh, Pittsburgh, Penna.* Rabbits and dogs were fed a standard prepared laboratory diet to which has been added daily 1 gm/kg. of body weight of cholesterol dissolved in ether. Following the development of hypercholesterolemia acute renal damage was produced by the administration of uranium or a nephrotoxic serum. The animals were killed from 3 days to 3 weeks later and approximately 80% of the rabbits and 60% of the dogs demonstrated aortic atheromatosis. This was in sharp contrast to the control group of animals without renal damage in which the incidence of aortic atheroma was extremely low.

Nature of the breathing impairment in anthracosilicosis. HURLEY L. MOTLEY and LEONARD P. LANG (by invitation). *Dept. of Medicine, Jefferson Medical College and Cardio-Respiratory Laby., Barton Memorial Division of Jefferson Hospital, Philadelphia, Penna.* Physiological studies were made on more than 150 anthracite coal miners with respiratory complaints after prolonged exposure to dust and the breathing impairment was classified as follows: *1*) decreased ventilation (maximal breathing capacity and vital capacity); *2*) increased residual air (degree of emphysema expressed quantitatively as residual air precentage of total lung volume); *3*) disproportion between alveolar perfusion and ventilation (inadequate alveolar blood flow and reduced O_2 removal from inspired air and CO_2 elimination in expired air); *4*) impaired distribution (unequal alveolar aeration). Multiple involvement may occur contributing to the breathing impairment. Approximately $\frac{2}{3}$ of the patients had a significant degree of emphysema (residual air 35–70% of total lung volume). Vital capacity and maximal breathing capacity were of limited value in appraising the impairment in patients with little or no emphysema. Hyperventilation may be adequate as a compensatory mechanism for the emphysema in the early stages, in fact until loss of elasticity of the lungs and chest wall lowers the dyspnea level restricting ventilation. In most instances the mean pO_2 gradient between the alveoli and the arterial blood was increased, even in the absence of a significant degree of emphysema and reduction in ventilation. Studies with high and low levels of oxygenation indicate that the increased pO_2 gradient is primarily a difficulty of distribution and not of diffusion through the pulmonary membrane. The x-ray stage of silicosis was poorly correlated with functional change. No single test was satisfactory for correctly evaluating the breathing impairment.

The clotting mechanism in relation to protamine sulfate and heparin. PETER V. MOULDER, BURTON J. GROSSMAN and MARGARET H. SANDERSON (introduced by J. GARROTT ALLEN). *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* The blood of normal humans and dogs made incoagulable with a standard amount of heparin (Abbott) can be brought to coagulation with a remarkably constant amount of protamine sulfate (Lilly). The reverse of such a procedure, that is, adding increasing amounts of heparin to blood, does not have a sharp end point and the time factor cannot be standardized. The latter method is extremely sensitive to even minimal changes in the thrombocyte level, prothrombin efficiency, etc., whereas the back titration with protamine is independent of the thrombocytes and, within usable limits, is affected by only profound changes in prothrombin level and by hemophiliac blood. Humans and animals with a coagulation defect, characterized by an increased amount of protamine necessary to bring about coagulation of

heparinized blood, usually have a moderately increased clotting time, and only rarely is it incoagulable. The blood is unusually dark and in most instances gelation precedes coagulation. Normal color, 'normal clot', normal or nearly normal clotting time and a decrease to or toward normal of the amount of titrating protamine are produced by administration of toluidine blue or protamine sulfate. Strikingly dissimilar, however, are the protamine titration changes relative to the clotting time. Only 0.75 mg/kg. body weight of intravenous heparin is required to make the blood of a normal dog incoagulable (15 minutes after injection). The protamine requirement is increased when 1.0 mg/kg. of body weight or more is given, and 3–5 mg. must be injected to produce the changes similar to those seen in the patients and dogs.

Thalamic representation of tactile sensibility in the Macaque monkey. VERNON MOUNTCASTLE and ELWOOD HENNEMAN (introduced by PHILIP BARD). *Dept. of Physiology, Johns Hopkins Univ., School of Medicine, Baltimore, Md.* The thalamic terminations of afferent systems conveying cutaneous tactile sensation have been mapped by an application of the evoked potential technique. The responsive area is contained within the nuclei ventralis posterolateralis and posteromedialis. No responses were recorded from lateral, medial or anterior nuclear groups. The contralateral body surface is represented in nucleus ventralis posterolateralis in dermatomic sequence, the caudal segments being most lateral, followed by lumbar, thoracic, and cervical segments in successively more medial positions. Those portions of the body most heavily innervated by sensory neurons, the apices of the limbs, receive greatest volume representation in the thalamus. The enlargement of the thalamic zone for the lower cervical segments (hand) has separated the zone for the upper cervical segments (head and neck) from that for the trigeminal nerve.

The face and mouth are represented in nucleus ventralis posteromedialis. The pattern suggests a para-oral, segmental representation of the trigeminal fields, with lips and mouth placed medially. The intra-oral structures are represented bilaterally. In the most medial position is a small area for ipsilateral face. Responses to tactile stimulation of ipsilateral body and face have been recorded from points in and near the external medullary lamina, but a zone of thalamic transfer of impulses destined for the second somatic area of the cerebral cortex has not been located definitely.

Studies on the renal secretion of potassium in the dog. GILBERT H. MUDGE (by invitation), JAMES G. FOULKS (by invitation), ADELBERT AMES, III (by invitation), and ALFRED GILMAN. *Dept. of Pharmacology, College of Physicians and Surgeons, Columbia Univ., New York City.* Potassium (K) clearances (CK) greater than simultaneous creatinine or thiosulfate clearances (CF) are considered evidence that a part of urinary K results from renal tubular secretion. CK/CF ratios over unity are most readily obtained by the intravenous infusion of K salts, a procedure which yields average ratios of approximately 1.3, occasionally rising higher. The intravenous infusion of hypertonic solutions or urea and sodium chloride also promotes the excretion of K. During water diuresis CK/CF remains low. A variety of drugs known to affect renal function has been studied for their effects on K excretion. Mercurial diuretics were the only agents to possess a significant action. These sharply diminished the excretion of K during K administration and during states of hypertonicity, and slightly elevated CK/CF during water diuresis. The effects of mercury were rapidly reversed by BAL. Although it is not possible from the data thus far obtained to determine the exact contribution of the secretory process to the excretion of K, the available results may be explained by the hypothesis that tubular secretion is a response to hypertonicity or to elevated cellular K following K infusion and that the action of Hg^{++} is to depress this process. If this hypothesis is correct it follows that a major portion of excreted K results from secretion.

Polarographically determined protein index. Nature of the cobalt-catalyzed reaction of cysteine. OTTO H. MÜLLER. *Dept. of Physiology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* In continuation of the studies of the 'protein index' (Müller and Davis, *Arch. Biochem.* 15: 39, 1947) it became necessary to know more about the nature of the electrode reaction which produces the catalytic wave of sulfhydryl groups in the presence of a buffered cobalt solution. Consequently cysteine was polarographed over a large range of concentrations in solutions containing 0.1 N NH_4OH, 0.1 N NH_4Cl and several different concentrations of $CoCl_2$. The catalytic wave was observed at cysteine concentrations as low as 2×10^{-7} M. Its height is proportional to the surface area of the mercury drop but it is not a linear function of the Co^{++} concentration. It plotted against the logarithm of the cysteihe concentration, it yields an S-shaped curve, typical of catalytic reactions. At higher cysteine concentrations a new wave was discovered which just precedes the above catalytic wave; it usually overlaps with it at lower concentrations. The height of this new wave is proportional to the concentration of Co^{++} ions and to the sixth root of the drop time, which suggests a type of diffusion current; its relationship to the cysteine concentration is more complex. From this and other evidence it is concluded that the first mentioned wave is produced by the catalytic action of the cobalt amalgam which is formed at the dropping mercury electrode, whereas the new wave is produced by the action of a

cobalt-cysteine complex which diffuses to the dropping mercury electrode.

Cardiac effects of aliphatic sympathomimetic amines in dogs under cyclopropane anesthesia. QUILL R. MURPHY (by invitation) and WALTER J. MEEK. *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison, Wis.* Cardiac irregularities produced by a series of aliphatic sympathomimetic amines have been observed in dogs under 30% cyclopropane-in-oxygen anesthesia. Each dose of drug was diluted to 5 cc. with distilled water and was injected intravenously at a constant rate within a period of 50″. Electrocardiographic records were taken. The results from 4 drugs of the series appear in the table. Tuamine, 2-amino-4-methyl hexane and 2-methylamino-1-cyclopentyl propane were given in dosages approximately equivalent to the pressor action of .01 mg. of epinephrine per kg. weight of the dog (Chen). The epinephrine pressor equivalent of EA 83 was not determined but a 10 mg/kg. dose of this drug should be sufficient to give a maximum pressor response (Jackson).

DRUG	DOSE	NO. DOGS GIVEN DRUG	EKG FINDINGS AND NO. OF DOGS IN WHICH EACH APPEARED			
			S-A Tachy.	No vent. irrge.	Vexs. only	Vent. tachy.
	mg/kg.					
2-methylamino-6-hydroxy-6-methyl heptane (EA83)	10	16	15	1	0	15
	5	15	13	5	5	5
2-amino heptane (Tuamine)	3	15	13	6	6	3
2-amino-4-methyl hexane	3	11	10	6	3	2
2-methylamino-1-cyclopentyl propane	2	6	6	6	0	0

The results indicate that regardless of a rise in blood pressure the sympathomimetic amines of the aliphatic series are apt to cause a S-A tachycardia of 215 or over. As judged by the frequency of ventricular tachycardia a 10 mg/kg. dose of EA 83 stimulates the lower centers of cardiac automaticity to about the same degree as does the standard test dose of epinephrine in dogs under cyclopropane. Ventricular tachycardia occurred following the injection of tuamine and 2-amino-4-methyl hexane much less frequently than following epinephrine. There was no evidence of ventricular stimulation by 2-methylamino-1-cyclopentyl propane even when the drug was given in dosages 50% in excess of its calculated epinephrine pressor equivalent.

Lipid metabolism in arctic Alaskan birds. X. J. MUSACCHIA (introduced by CHARLES G. WILBER). *Biological Laby., Fordham Univ., New York City.* Both the Old Squaw, *Clangula hyemalis*, and the Red Phalarope, *Phalaropus fulicarius*, regularly migrate to Arctic Alaska each year. They are extremely abundant, during the summer months, in the Point Barrow region. The birds were hunted and killed with shotguns. The internal organs (liver and kidney) were removed immediately after death. The organs were weighed and placed in vials containing Bloor's alcohol-ether mixture. Alcohol-ether extracts of the tissues were made. Detailed fat analyses were conducted, using standard biochemical procedures.

The average amounts of lipids (expressed as percentage of wet weight) for the 2 birds are shown in the following table. Each number is the mean of values obtained from 10 individuals.

	CHOLESTEROL TOTAL	CHOLESTEROL ESTERS	LIPID PHOSPHORUS	FATTY ACIDS
Old Squaw				
Liver	0.42	0.27	0.15	3.0
Kidney	0.29	0.12	0.12	2.8
Phalarope				
Liver	0.35	0.15	0.22	4.5
Kidney	0.32	0.14	0.15	2.3

Free cholesterol values, obtained by subtracting esters from total, are as follows: Old Squaw, liver 0.15, kidney 0.17; Phalarope, liver 0.20, kidney 0.18. Values for phospholipid expressed as lecithin are as follows: Old Squaw, liver 3.8, kidney 3.0; Phalarope liver 5.5, kidney 3.0.

The relationship of cholesterol/phospholipid, free cholesterol/total cholesterol and cholesterol/fatty acid were calculated:

	CHOLESTEROL PHOSPHOLIPID	FREE CHOLESTEROL TOTAL CHOLESTEROL	CHOLESTEROL FATTY ACID
Old Squaw			
Liver	1.8	0.37	0.14
Kidney	2.4	0.58	0.10
Phalarope			
Liver	1.6	0.57	0.08
Kidney	2.1	0.56	0.13

Mechanism of the toxic effects of inhibitors of acetylcholine-esterase. DAVID NACHMANSOHN. *Dept. of Neurology, College of Physicians & Surgeons, Columbia Univ., New York City.* A great variety of facts have been described supporting the assumption that the toxic effects of inhibitors of choline-ester-splitting enzymes have to be attributed to the action upon the acetylcholine-esterase. Many apparent contradictions reported in the beginning have been satisfactorily explained. However, great differences exist between the toxic signs produced by the various compounds in spite of the fact that the enzyme inhibition is reponsible in all instances. A close analysis has revealed a great

number of factors upon which these differences depend, *in vitro* as well as *in vivo*. Even working with the virtually pure enzyme solution, the reaction rate depends at least on three variables: enzyme, inhibitor and substrate. Variations of the concentration of each of these three components affect markedly the inhibitory effect. Considerable differences are observed between reversible and irreversible inhibitors, but even compounds belonging to the same group may show entirely different behavior. A few examples will be discussed. Still more factors determine the effect *in vivo*. Of special importance is the structural barrier surrounding the axons. Lipid insoluble compounds act only upon the synapse. But between lipid soluble inhibitors exist interesting differences. Some physiological implications of these observations will be discussed.

The spread of excitation and the order of depolarization in the human heart. L. H. NAHUM and H. M. CHERNOFF (by invitation). *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* To determine the nature of VR, VL and VF in man it is necessary to know the 'proximal' and 'distal' zones of each lead. This information was obtained by projecting solid cones from the position of each electrode upon the heart, the area subtended being considered the proximal zone. The position of the heart was taken from X-ray views obtained at various angles. A geographic map was then constructed, showing the overlapping of the proximal and distal zones. By analyzing the positions of the beams from moment to moment in the simultaneously recorded QRS complex of each lead, the order of arrival of the excitation process at the various segments of the heart was determined.

The region first to receive the excitation process was lower anterior septum. Excitation then spread rapidly to involve anterior and posterior right ventricle, then to left apex and left posterior ventricle. Excitation terminally involved a region of the right anterior ventricle (conus area). This order of spread corresponds to that already described in the dog and agrees with observations made upon the exposed human heart. The order of repolarization was determined from analyzing the position of the ST-segment and direction of the T-wave in unipolar extremity leads and in simultaneously recorded CV1 to CV6. It was found that the apical and diaphragmatic region of the heart is the site of fastest repolarization.

Nitrogenous excretion in Colpidium campylum. ROLAND M. NARDONE (introduced by CHARLES G. WILBER). *Biological Laby., Fordham Univ., New York City.* Wilber and Seamen (*Biol. Bull.*, 93: 214, 1947) found that Colpidium campylum can synthesize fat from protein. The following investigation was undertaken in an attempt to ascertain the nature of nitrogenous excretion in *Colpidium campylum* when grown on a protein medium (proteose-peptone). Quantitative analyses for ammonia, in pure, sterile cultures of colpidia, were made using the permutit method (*J. Biol. Chem.* 29: 329, 1917). No ammonia was found 24 hours after innoculation. A straight line increase in ammonia with time was obtained from the 2nd day after innoculation to the 14th day after innoculation. The mean population reached a maximum of approximately 68,000 organisms per ml. of culture on the 9th day after innoculation. The population then decreased gradually to approximately 41,000 colpidia per ml. on the 14th day after innoculation. The results shown below are mean values of 5 analyses each day.

NO. DAYS AFTER INOCULATION	POP./ML.	AMMONIA N
		γ/ml.
1	5,500	0.00
2	17,025	0.71
3	18,450	1.43
4	46,620	4.28
5	52,500	6.09
6	61,200	7.85
7	63,855	9.10
8	62,550	8.20
9	67,965	10.00
10	50,110	13.85
11	53,100	13.20
12	43,240	16.40
13	40,350	18.05
14	40,890	17.90

Using a qualitative spot test (Feigl), no uric acid was detected throughout the experiment. Urea, however, was produced during the logarithmic phase of growth.

Effects of eserine and pilocarpine on the parasympathetically denervated iris of the cat. ENID A. NEIDLE (introduced by WALTER S. ROOT). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* According to Anderson (1905) the iris of the cat which, immediately after removal of the ciliary ganglion, responds to eserine soon ceases to do so and remains unresponsive. There is then a gradual return of the eserine response in the denervated sphincter. This has been taken as evidence for nerve regeneration. In 5 cats in which one ciliary ganglion had been removed and which showed a return of responsiveness to eserine, the oculomotor nerve was sectioned intracranially. Ten days later, the response of the sphincter was tested by instilling 0.4 mg. of eserine salicylate into the conjunctival sac. In every instance there was complete or partial loss of respone. This supports the belief of Anderson (1905) that preganglionic cholinergic fibers can replace postganglionic cholinergic fibers. Instillation of 0.04 mg. of pilocarpine nitrate in the conjunctival

sac produces marked constriction of the ganglionectomized iris but has no effect on the normal iris. The sensitivity of the denervated iris to pilocarpine diminishes slightly but significantly with time after operation. Section of the oculomotor nerve, some time after removal of the ciliary ganglion, is followed by a complete or partial restoration of sensitivity to topically applied pilocarpine. It is suggested that the decrease in sensitivity to pilocarpine may be related to the occurrence of regeneration to the denervated sphincter of the iris.

Effect of hydration on the survival of fasting rats. DOROTHY NELSON (introduced by A. C. IVY). *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Rats deprived of food dehydrate themselves even in the presence of adequate water. There is considerable evidence in support of the hypothesis that rats ingest any available material that is of benefit to them. In sharp disagreement, a growing literature indicates that in many situations rats fail to make advantageous selection of foods. Is the voluntary dehydration of the fasting rat protective or detrimental? To clarify this point we have compared the survival of several groups of mature females. With no food or water the rats lived about 11 days. The injection of 7.5 cc. of 0.6% NaCl solution every 12 hours prolonged life about 3 days, a significant increase. Similar injection of 0.9% NaCl solution had no effect on survival, though less weight was lost than in the control groups. It appears from this, that the rat's refusal to drink in the absence of food, does not represent an attempt to maintain an optimal water balance, but probably results from a break down of the mechanisms for fluid adjustment.

Effect of elemental iodine and iodocasein in hypothyroidism induced by methyl thiouracil. WARREN O. NELSON and EILEEN B. HINES (by invitation). *Dept. of Anatomy, State Univ. of Iowa, Iowa City, Iowa.* Young male and female rats weighing 40–50 gm. were placed on a stock diet containing 0.2% methyl thiouracil. After 30 days the animals in each experiment were divided into 3 groups. One continued on the methyl thiouracil diet; a second received the same diet with 0.033% iodocasein; while the third received the same diet, but in addition received 4.0 or 6.0 mg. elemental iodine daily by subcutaneous injection. The second phase of the experiment was continued for 30 or 60 days. Control animals received the regular stock diet throughout the experimental period. For males in the longer experiments the averages for final body weight and total length were respectively: normal controls, 375 gm. 41 cm.; methyl thiouracil only, 95 gm., 28.5 cm.; methyl thiouracil and iodine, 175 gm., 33 cm.; methyl thiouracil and iodocasein, 252 gm., 38 cm. The animals which received methyl thiouracil only showed markedly reduced testicular function, both gametogenic and endocrine. The adrenals, thymus, spleen, liver, heart, submaxillary glands and kidneys all showed marked reduction in weight as compared to the controls. However, all of these except the thymus corresponded to the reduction in body weight. Iodine induced definite improvement in testicular function and in organ weights, but was less efficacious than iodocasein. Females of the several groups showed the same trends for body weight, body length, ovarian function and organ weights. Estrous cycles were markedly improved by both iodine and iodocasein. Determinations of plasma protein-bound iodine revealed high levels for animals which received iodine or iodocasein.

Transmission spectra of the human ear (pinna and lobe) at arterial occlusive pressures. J. F. NEVILLE, JR., J. O. ELAM, K. SUGIOKA, and A. ROOS (introduced by H. L. WHITE). *Laby. of Applied Thoracic Physiology (Surgery) and Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.* Factors related to the accuracy of the oximeter have been investigated by spectrophotometry of the intact human ear. Instrumentation consisted of a Littrow type monochromator (resolution of 80 Å), S-4 and S-1 response phototubes, AC amplifier, and continuous ink recorder. The monochromatic beam was directed via quartz to the ear of the recumbent subject. The recording spectrophotometer automatically scanned the spectrum between 4000 and 11000 Å. Ear tissue was compressed with translucent rubber diaphragm by pneumatic inflation to 200 mm. Hg. Spectra were calculated as percentage absolute transmission ($\% T = \frac{I_t}{I_o} \times 100$, where $I_0 =$ phototube response). Data obtained in 10 male and 2 female normal white subjects indicated: *1*) the bloodless pinna and lobe transmissions were zero at wavelengths shorter than 5100 Å; *2*) the maximal transmissions of the bloodless pinnae were uniformly at 8800 Å; *3*) the maximal transmissions of the bloodless lobes covered a broad spectral band from 6500 to 10000 Å *4*) the absolute transmissions of the bloodless pinnae averaged 15% between 6000 and 10000 Å while comparable values for the lobes averaged 26%; *5*) the spectra of the bloodless pinnae indicated marked light scattering between 8800 and 5100 Å (decreasing transmission with decreasing wavelength) while this effect was much less evident in the lobe spectra; and *6*) the absorption band of water between 8500 and 9800 Å was evident particularly in the pinna spectra. The lobe of the ear appears to offer optical advantages superior to the pinna as the site for oximetry.

Effect of diets containing certain tissues on liver regeneration in the rat. E. NEWMAN (by invitation) and A. C. IVY. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Previous studies in this laboratory had shown that liver regeneration in partially hepatectomized rats was more rapid in rats fed whole cooked liver or

dried whole liver than in those fed casein or beef heart. The growth promoting factor was shown not to be in crude liver extracts. In order further to analyze this effect diets containing certain organs other than liver were fed to partially hepatectomized rats. The organs studied included spleen, kidney and duodenum. None of these organs were equal to liver in promoting liver regeneration. Studies were also made using nucleic acid or uracil supplements to a standard synthetic diet. Nucleic acid (derived from yeast) was as effective as whole liver in promoting liver regeneration. Uracil had no effect.

A 'vertical tube' method for the study of pressure-flow relations in vascular beds. J. NICHOL (by invitation), F. GIRLING (by invitation) and A. C. BURTON. *Dept. of Biophysics, Univ. of Western Ontario, London, Canada.* In the perfused preparation, as the isolated rabbit's ear, a vertical small-bore glass tube is connected (as a 'T') to the arterial cannula through which perfusion is made, while in the 'intact' preparation, as the hind-limb it is connected to a T-cannula inserted into the artery. In the latter case, the fluid in the vertical tube is separated from the flowing blood by a slack rubber membrane. The liquid in the tube stands at a height giving the pressure in the artery at that point. The artery, or tube from the perfusion bottle, is then clamped on the high pressure side of the T. The circulation in the preparation then proceeds, maintained by 'bleeding' from the vertical tube, at a steadily diminishing driving pressure. The height of the vertical column is plotted against time, giving an exponential-like curve. Tangents drawn to the curve at specific pressures give the flow through the vascular bed at any pressure. It can be shown that the length of the sub-tangent is a direct measure of the resistance to flow. Complete pressure-flow relations are thus obtained. These agree with those obtained by altering the pressure head and measuring flow by a micro-flow meter, but the vertical tube method is much more convenient and very accurate at low pressures and flows, where theoretical interest is greatest.

Time course of failure of the visual pathway in rabbits during anoxia. WERNER K. NOELL and HERMAN I. CHINN (introduced by HARRY F. ADLER). *USAF School of Aviation Medicine, Randolph Field, Texas.* In order to determine the factors responsible for the sequence of anoxic failure, potentials resulting from illumination of the eye and from electrical stimulation of the optic nerve were recorded from different parts of the visual pathway. Amplitude and timing of these responses were measured during nitrogen breathing. The curves for the decline in responsiveness thus obtained reveal, as one factor, the effectiveness of a decreased excitability on differently organized neuronal systems. Complete irresponsiveness at the surface of the striate area to either photic or electrical stimuli was reached after 88 seconds of nitrogen breathing (blood was oxygen free within 30 to 45 seconds). The immediate postsynaptic potentials from the geniculate disappeared after 113 seconds. Potentials of the optic tract to strong photic stimuli became undetectable after 256 seconds and to maximal electrical stimuli after 293 seconds. One portion of the b-wave of the electroretinogram decreased simultaneously with the optic tract potentials; 25 minutes were required until all positive components of the electroretinogram had disappeared. A small negative electroretinogram persisted for many hours. All figures represent mean values. Intravenous administration of iodoacetate quickly abolished retinal excitability during air breathing. Neither the anoxic decline of cortical responsiveness nor the failure of the optic nerve to direct stimulation was hastened. Lethal doses of fluoride did not specifically affect retinal excitability immediately after injection. However, during subsequent anoxia all positivity of the electroretinogram disappeared more rapidly, while the survival times of cortex and optic nerve were not decreased.

Incorporation of glycine in the tissues of acutely starved rats. T. R. NOONAN, K. I. ALTMAN, G. W. CASARETT, and K. SALOMON (introduced by WILLIAM F. BALE). *Dept. of Radiation Biology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* The effects of acute starvation upon the incorporation in tissues of glycine, labelled in its alpha-carbon atom with C_{14}, have been studied in the rat. One microcurie of labelled glycine (specific activity 1.83 μc/mg.) was injected intravenously into normal rats and into rats which had been starved for 5 to 7 days; 24 hours after injection, the animals were killed and tissue samples secured for analysis of C_{14} content. Samples from the liver, gastrointestinal tract, kidneys, testes, and brain of animals starved for 5 or 7 days showed a markedly higher radioactivity per gram than corresponding samples from control rats. On a unit weight basis, the muscles of starved animals showed lower C_{14} concentration than control animals, this effect being more marked after 7 days of starvation. When calculated on the basis of percent of injected C_{14} activity found per total organ, the starved animals showed a pattern of decreased incorporation in muscular tissue with an unchanged or even increased incorporation in visceral tissues.

Time-dose study of biochemical responses of rats to x-radiation. N. NORTH (by invitation) and L. F. NIMS. *Brookhaven National Laby., Upton, L. I., N. Y.* A time-dose study of biochemical responses of the adrenal-pituitary system in fasted rats following whole body x-radiation indicates a pattern of changes comparable to that following other stresses. Male albino rats were subjected to radiation doses of 500 to 2000 R at a rate of 50 R per minute and sacrificed at periods of time ranging

from 3 to 48 hours following exposure. Analyses of tissue constituents show an immediate and progressive increase in liver glycogen and no change in testis glycogen. There is a simultaneous decrease in the adrenal and liver cholesterol but little change in the testis cholesterol and a late increase in the plasma cholesterol. The adrenals also show an initial decrease in ascorbic acid content with a subsequent return to normal, while little variation from the unirradiated controls is seen in pituitary, liver or testis ascorbic acid. Thrre are no evident effects of radiation on total body, adrenal or pituitary weights in fasted animals.

Acclimatization to histotoxic anoxia. DAVID W. NORTHUP and ROSS BELL, JR. (by invitation). *Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown, W. Va.* In order to determine whether acclimatization to histotoxic anoxia occurs in a manner similar to that seen during anoxic anoxia, the red cell count, reticulocyte count, and hemoglobin concentration of the blood of three rabbits were followed during the administration of potassium cyanide. Determinations were made daily during an 11-day control period and then KCN was administered subcutaneously in doses ranging from 1.0 to 3.1 mg/kg/day, the dose being increased as the animals developed tolerance. (Sufficient cyanide was given to produce distress.) The average red cell count of the 3 rabbits rose from 4.97 million during the control period to 5.37 million during the period of cyanide administration, significant at the 0.1% level; the hemoglobin from 10.95 gm. to 11.92 gm. significant at the 0.1% level; the reticulocyte count from 1.0% to 2.3%, significant at the 0.1% level. The results indicate that the rabbit responds to histotoxic anoxia by increased activity of the bone marrow.

Inside the cell. Part I: Enzymes in intracellular chemistry (Motion picture). ALEX B. NOVIKOFF (introduced by F. J. SICHEL). *Dept. of Pathology, Univ. of Vermont College of Medicine, Burlington, Vt.* Produced by the United States Army Medical Illustration Service, under the supervision of the Surgeon General, this film is intended for teaching professional medical personnel of the Services and civilian medical and university students. It suggests the importance to medical practice of a knowledge of enzymes and cell metabolism. After briefly sketching the historical development of our knowledge of enzymes, the film deals in detail with the known steps of glucose metabolism, including glycolysis, the tricarboxylic acid cylce, hydrogen transport via the cytochrome system, and the phosphate or energy cycle. It suggests points of contact of the metabolism of carbohydrates and other foodstuffs, and it indicates the relation of vitamins and minerals to enzyme structure. Most of the film is in color animation. There are also live action demonstrations of several sections, photographed in the laboratories of the individuals mentioned. J. B. Sumner shows the essential steps in the crystallization of urease. Otto Meyerhof demonstrates the need of DPN and ATP in glycolysis. Severo Ochoa shows the enzymatic decarboxylation of oxalosuccinic acid and the oxidation of isocitric acid. Carl F. Cori and Gerty Cori demonstrate the *in vitro* synthesis of glycogen and starch. Carl F. Cori and M. E. Krahl show the effect of insulin and adrenal hormone on the uptake of glucose by diaphragm muscle. Van R. Potter demonstrates the low succinic dehydrogenase activity of tumor homogenates as compared with those of normal organs.

Response to intravenous injection of small quantities of insulin. JOHN A. NUETZEL (by invitation) and NORMAN S. OLSEN. *Dept. of Internal Medicine and the Oscar Johnson Inst., Washington Univ. School of Medicine and Barnes Hospital, St. Louis, Mo.* A modified intravenous insulin tolerance test, using $\frac{1}{10}$ unit of insulin/kg. body weight, was performed in a series of normal, hypertensive, and other patients. Blood sugar levels were determined at 0, 15, 30 and 60 minutes after intravenous injection, by the method of Nelson. The data were analyzed on the basis of the percentage fall from the fasting level. Certain clinical groups were resistant to the action of intravenously administered insulin in that they showed significantly less fall in 15 and 30 minutes than did the normal group. These groups consisted of patients with malignant hypertension, with hypertension and severe complications (such as stroke and heart failure), with advanced neoplastic diseases, liver diseases, collagen diseases, and psychosomatic disorders. Resistance was also found, as expected, in active infections and certain endocrine disorders. Hypertensive patients having an endocrine or neurogenic basis and not in a malignant phase were not resistant.

Blood pressure after nephrectomy. ERIC OGDEN, ELEANORE TRIPP (by invitation) and GEORGE CONSTANT (by invitation). *Dept. of Physiology, Univ. of Texas Medical Branch, Galveston, Texas.* Grollman reported occasional elevations of blood pressure in rats and dogs after unilateral nephrectomy. In the present group of 5 well-trained dogs series of measurements were made before and 5 months after removal of the left kidney. The table indicates the average values for the individual dogs.

DOG	BEFORE NEPHRECTOMY		AFTER NEPHRECTOMY	
	Syst./Diast.	P. rate	Syst./Diast.	P. rate
1	165/80	115	194/105	100
2	168/83	90	188/107	101
3	133/60	108	166/ 80	86
4	139/59	95	167/ 95	95
5	165/81	150	171/ 97	91

Blood pressure was computed from records made without anesthesia by puncture of the femoral artery with the needle connected to a glass spoon manometer with optical recording; the pulse counts were computed from the same records. It is believed that the lowered pulse rates after nephrectomy are indications that the dogs were better trained. The elevation of both systolic and diastolic pressures in each animal considered separately was statistically examined and found unlikely to be due to chance. That such an occurrance would appear in 5 consecutive animals is over-whelmingly likely to be due to something other than chance even though the changes are small and only two of the dogs showed blood pressure levels which might be considered mildly hypertensive.

Treatment and prophylaxis of experimental hypertension with amines. E. A. OHLER (by invitation), G. E. WAKERLIN and G. A. ALLES (by invitation). *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago, Ill.* Paredrine HBr has been previously reported (*Federation Proc.* 7:87, 1948) to be effective therapeutically in experimental renal hypertension. Since our last report 3 neurogenic (debuffered) hypertensive dogs have been treated with 4 mg/kg of Paredrine orally for periods of 3 to 6 months. Two neurogenic dogs showed excellent reductions in blood pressure; one, a good response. Of 5 dogs treated prophylactically with 4 mg/kg of Paredrine orally daily for periods of 3 to 6 months, all showed a rise in blood pressure following bilateral renal artery constriction. Two animals exhibited rises of 55 mm. Hg; one dog developed malignant hypertension and died, and the remaining 2 showed rises of 20 mm. Hg or more. The following compounds, closely related chemically to Paredrine, have failed thus far to exhibit any antihypertensive activity: 2-amino-1-cyclohexylpropane, levoamphetamine, ephedrine, hydroxy-tyramine, hydroxy-paredrine, and dl-l-parahydroxyphenyl-3-aminobutane. Tyramine in a dosage of 2 mg/kg IM daily produced a modest fall in the blood pressure of 1 renal hypertensive animal. Of 3 renal hypertensive dogs treated with Paredrinol (dl-l-parahydroxyphenyl-2-methyl-aminopropane) in a 4 mg/kg dose orally only one showed a reduction in blood pressure. Tuamine (2-aminoheptane) at a dosage level of 4 mg/kg IM has proved ineffective in 2 hypertensive animals.

Diuretic response of normal and edematous dogs to intravenous administration of succinate and fumarate sodium salts. ELIZABETH O'LEARY (by invitation) and SAMUEL A. CORSON. *Dept. of Physiology, School of Medicine, Howard Univ., Washington, D. C.* Hypertonic solutions (1.4M) of disodium succinate and fumarate salts were administered intravenously to trained unanesthetized dogs by means of a constant infusion pump at the rate of 2.5 ml/min., injecting a total dose of 10 ml/kg. of body weight. Such an infusion increased the rate of urine flow from an average control value of 0.4 ml/min. to an average value of 4.2 ml/min. for a period of about 300 minutes. The net quantity of fluid lost by the animals as a result of the administration of these salts (after subtracting the quantity of fluid injected and the quantity of urine that would normally have been excreted) was on the average 67 ml/kg. body weight. Administration of these salts to dogs with experimental hypoproteinemic edema (produced by a combination of massive plasmaphereses and a low protein diet) led to a net average increase in urine output of 76 ml/kg. body weight. We must therefore conclude that the diuretic responses of dogs with hypoproteinemic edema to the intravenous injection of succinate and fumarate salts are of the same order of magnitude as (or possibly greater than) those of normal animals.

Graphic measurement of arterial pressure in the intact rat. F. OLMSTEAD and O. GLASSER (introduced by A. C. CORCORAN). *Research Division of the Cleveland Clinic Foundation and the Bunts Institute, Cleveland, Ohio.* The method previously described (*Federation Proc.* 7:88, 1948) for measurement of systolic pressure from the foot pulse of rats had been modified for greater accuracy and convenience. A light weight pickup has been developed which consists of 2 minature coils separated by a small layer of sponge rubber. This device is readily attached to the front or hind foot of the rat and substitutes for the strain gauge previously employed. A frequency discrimination circuit is used to transfer the pulse into electrical change. For greater compactness and accuracy in recording cuff pressure, a Selsyn generator and motor are used. The generator is driven by a large metal bellow. The motor is mounted in the Brush ink oscillograph and drives a recording pen. Seventy-five simultaneous optical recordings were made of carotid pressure by a Hamilton manometer and of foot systolic pressure as indicated from the foot pulse. The mean deviation of indicated pressure in the hind foot from that in the carotid artery was —5.4 mm. Hg. Variation around this mean was ± 3.5 mm. Hg. These measurements were made over a range of systolic pressure from 52 to 170 mm. Hg.

Effect of Intrapulmonic and intrathoracic pressure variations on left atrial pressure. D. F. OPDYKE and G. A. BRECHER (by invitation). *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, Ohio.* Mechanical factors of extra-cardiac origin which exert pressure on the heart chambers become of great importance in determining pressure-flow gradients in the presence of intra-cardiac shunts. If these extraneous forces do not affect all cavities equally it is possible that their effect may be directly opposite to that

desirable for physiologic compensation. Experiments have been undertaken to assess the effects of two of these factors, intrapulmonic and intrathoracic pressure, on left atrial pressure, since the pressure variations in this heart chamber have been so little studied.

Positive pressure inflation of the lungs in an open chest dog results in an increase of both right and left atrial pressure with a decrease in aortic pressure. This effect is intensified by maintenance of the lungs in the inspiratory position for a few seconds. Right pressure increases considerably more than left. Ventral traction on the pericardium, lung roots, aorta, and other thoracic structures does not reproduce this effect. Experiments dealing with the effect of intrathoracic pressure changes and with the effects of positive pressure breathing on left atrial pressure in the closed chest dog are described and the results presented.

Diuretic effect of adrenal cortical hormones in mildly hydrated and dehydrated animals. C. M. OSBORN and W. J. EVERSOLE (introduced by ROBERT GAUNT). *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.* Experiments were made to determine the conditions under which the diuretic action of adrenal cortical hormones could be demonstrated in intact rats. In previous experiments with water given *ad lib*, desoxycorticosterone acetate (DCA) caused a diabetes insipidus-like state (Selye and Bassett); this condition was intensified by addition of salt to the diet (Corey and Britton; Ragan *et al.* Richter). When large multiple doses of water were force-fed, both DCA and cortical extract enhanced markedly the diuretic rate and prevented water intoxication (Gaunt). The possibility exists that such effects are manifested only in the presence of high water loads induced either indirectly by salt retention or directly by water administration. Forty-eight fasted male rats were given a single hydrating dose of water by stomach tube (4 ml/100 cm.^{2} of body surface; average dose = 12 ml.). One group received 5 mg. DCA one-half hour before water was given; 90 minutes later they had excreted 11% more urine than controls, a result similar to that following chronic DCA treatment (Selye and Bassett). With same water dosage, another group received 4 ml. of Upjohn's adrenal cortical extract (alcohol removed), force-fed as a portion of the water dose. These animals excreted 31% more urine than saline-treated controls. Forty-eight rats were deprived of food and water for 20 hours. Beginning at the 13th hour, part of the animals were given DCA at intervals until dosage totaled 4 mg. and others received a total dosage of 4 ml. of cortical extract. With such dehydration and acute treatment, DCA had no effect on urine volume, but the cortical extract doubled urine output. It appears, therefore, that the diuretic action of DCA may be somewhat dependent on water load, while cortical extract enhances diuresis under all observed conditions of water balance.

Application of Gray's theory of respiratory control to the hyperpnea produced by 'passive' exercise. ARTHUR B. OTIS. *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Movements of the limbs produced by external forces cause an increase in the minute volume of ventilation as, has been observed by several previous investigators. Such movements, however, are not entirely passive but produce an increased oxygen consumption. Continuous measurements of the alveolar gas tensions make possible the estimation of the ventilatory stimulus due to the limb movements *per se* by application of Gray's multiple factor theory. Such calculations show that the stimulus from such movements is enough to produce a ventilation increment 100% to 150% of the resting ventilation.

Anemia producing properties of hexahomoserine and the antagonizing effect of lysine. EDOUARD PAGÉ and R. GINGRAS (by invitation). *Depts. of Nutrition and Biochemistry, Medical School, Laval Univ., Quebec, Canada.* Hexahomoserine (α-amino-ϵ-hydroxycaproic acid) produces anemia and inhibits growth in the rat. It also causes a considerable rise in blood plasma amino nitrogen. Simultaneous lysine supplementation prevents the anemia, improves partially the growth rate but has no effect on the plasma amino nitrogen. These observations were made on rats receiving either a complete basal ration or one containing no lysine. Amino-adipic acid, a biological derivative of lysine, antagonizes neither hexahomoserine, nor lysine. Liver concentrates and folic acid are ineffective in the prevention of the anemia caused by hexahomoserine.

Mechanisms of the vascular action of tetraethylammonium chloride. IRVINE H. PAGE, RALPH PRINCE (by invitation) and JOHN J. REINHARD, JR. (by invitation). *Research Division and Bunts Institute of the Cleveland Clinic Foundation, Cleveland, Ohio.* Acheson, Moe, Lyons and Hoobler introduced tetraethylammonium chloride as a means of blocking autonomic ganglionic transmission to measure autonomic activity. Although we were unable to show a significant difference in response to TEA after renal hypertension developed, individual responses were so variable that blockade of ganglionic transmission probably was not the only action of TEA. Ninety-four normal dogs showed widely variant responses. Occasional 'normal' animals showed a rise in blood pressure rather than the more usual short rise followed by a fall. On repeating the doses, the responses became progressively less depressor and,

finally, pressor. Destruction of the spinal cord from C_6 caudad caused invariably a pressor response to every injection, and inactivation of the carotid sinus mechanism either did not change the response or somewhat augmented it. In 2 patients with severe central nervous system lesions pressor responses were observed with the initial dose. Bilateral nephrectomy done 2 days before testing usually accentuated the pressor component while bilateral adrenalectomy very moderately reduced it. Hepatectomy greatly increased the depressor action. TEA caused little or no rise in pressure after hepatectomy, hepatectomy-nephrectomy-adrenalectomy, or in animals with spinal cord destroyed, carotid sinus mechanism inactivated, kidneys and liver removed. The pressor response was abolished by prior administration of Dibenamine and Priscol sufficient to reduce or abolish the action of arterenol and reverse adrenalin.The response to TEA appears to be composed of 2 components: one dependent on blockade of autonomic ganglionic transmission; the other, dependent probably on liberation of an arterenol-like substance.

Observations on pulmonary lymph flow and edema. ROBERT PAINE (by invitation), FRANK A. HOWARD (by invitation), HARVEY R. BUTCHER (by invitation) and JOHN R. SMITH. *Cardiovascular Division, Dept. of Medicine, Washington Univ. School of Medicine, St. Louis, Mo.* Lymph flow from the lungs of dogs has been studied. Using Drinker's method, the pulmonary lymphatics were isolated, and intact, or open thorax preparations under positive pressure respiration, were made. The lung lymph was measured by cannalation or by catching the fluid on weighed pledgets. Obstruction of the pulmonary veins producing moderate elevation of venous tension and pulmonary congestion showed no effect on the rate of lymph flow. However, maximal pulmonary venous congestion results in increased lung lymph (Drinker). The reduction of plasma proteins to 2.5 gm. % or less by plasmaphoresis, and by the administration of Locke's solution, caused a marked increase in lung lymph flow. Furthermore, when pulmonary lymphatic drainage was curtailed by obstruction of the superior vena cava, a rise in lung lymphatic pressure occurred as noted by direct manometry of the lymphatic system. However pulmonary edema was not observed following any of these procedures. When lymphatic outflow was obstructed, the critical diminution of plasma proteins produced gross pulmonary edema. The effects of increased hydrostatic pressure and decreased osmotic pressure indicate the operation of Starling's hypothesis in the lung. The curtailment of lymph outflow due to peripheral venous hypertension from heart failure, in conjunction with pulmonary congestion or hypoproteinemia, may be important in the precipitation of pulmonary edema.

Variability and reliability of cardiac index estimations with the high-frequency, undamped ballistocardiograph. ROBERT M. PAINE (by invitation) and NATHAN W. SHOCK. *Section on Cardiovascular Diseases and Gerontology, National Heart Inst., Bethesda, Maryland, and the Baltimore City Hospitals, Baltimore, Maryland.* The ballistocardiograph used was horizontal, undamped, and had a natural frequency of 12.6 cycles/sec. when loaded with 150 lbs. The calibration was such that a force of 280 gm. applied to the bed caused a displacement of 1 centimeter on the photographic paper record. The largest and smallest complexes in each of 2 representative respiratory cycles of each tracing were selected independently by 2 observers and the average areas (mm.—sec.) under the I-waves and J-waves were inserted into Tanner's formula: stroke volume in cc. per minute = $\sqrt{21 + J\sqrt{C}}$. C represented the duration of a cardiac cycle in seconds. Cardiac index (L/min/M.^{2}) was estimated in the basal state, after a standard meal, at hourly intervals throughout a day of ordinary activity, and at 5-minute intervals, through a 40-minute period while resting supine. Ten young male hospital inpatients without cardiovascular disease and 10 young male laboratory personnel served as subjects. The entire routine was repeated on the inpatients the following day. A significant elevation of mean cardiac index (12%) occurred within ½ hour after eating which lasted 3½ hours. There was no difference between inpatients and laboratory personnel, in either the basal state or following ordinary activity, and there was no change through forty minutes of rest; similar results were obtained on the second day. The reliability of the cardiac index was high and variation in any case determination depended largely on the complexes chosen. No significant advantage was obtained by increasing the number of complexes measured or the number of observers.

Adrenal cortical hormone levels in blood following 'alarming stimuli.' K. E. PASCHKIS, A. CANTAROW and D. BOYLE. *Jefferson Medical College, Philadelphia, Penna.* The reaction of the adrenal cortex to 'alarming stimuli' was studied by assay of adrenal cortical hormone in peripheral arterial blood of dogs. Hormone levels were determined in plasma by the mouse liver glycogen method of Venning. It had been shown previously (*Fed. Proc.* 7 : 90, 1948) that levels in the arterial blood rise following the injection of ACTH or of adrenalin. A rise of cortical hormone in arterial plasma occurred within the first hour after subcutaneous injection of formalin. A surgical procedure, exposure of the adrenal gland under anesthesia, resulted in higher levels of cortical hormone in the adrenal vein blood than had been previously obtained in the unanes-

thetized, trained dog (London cannula.) Insulin injection was followed by an increase in cortical hormone, suggesting the participation of adrenal cortical hormone in counteracting insulin-induced hypoglycemia. Attempts to determine whether the effects of these stimuli were mediated by epinephrine (according to the theory of Long) led to an investigation of the action of Debenamine in this connection. Injection of Dibenamine itself was found to be an 'alarming stimulus', leading to an increase in blood levels of cortical hormone. The effect of sympathetic blockade by Dibenamine upon the response to formalin and insulin was studied.

Influence of a conditioning injection of estrogen on the hematologic and organ weight response to X-irradiation. HARVEY M. PATT, MARGUERITE N. SWIFT (by invitation), ROBERT L. STRAUBE (by invitation), ELLA B. TYREE (by invitation) and DOUGLAS E. SMITH. *Biology Division, Argonne National Lab., Chicago, Ill.* Estradiol benzoate (0.166 mg. I.M. in a single injection 5 to 15 days before exposure) greatly enhances resistance of mice to total body-X-irradiation. Progesterone and testosterone are ineffectual. To elucidate this phenomena we have studied the effect of estradiol on organ weights, blood count, and microscopic anatomy in control and irradiated mice. Estrogen increases adrenal weight by 35–40% 4 days after injection and accelerates thymic involution but has no appreciable effect on weight of spleen, inguinal nodes or kidney. Leukopenia with maximal depression around 10–14 days after injection has also been observed. Decreases are noted in lymphocytes and heterophils. Little change is seen in erythrocytes. Significantly, the maximal estrogenic protective effect is observed when mice are irradiated during their leukopenic period. Estrogen injection at the time of irradiation or 25 to 35 days before exposure does not protect. Radiation induced involution of spleen, thymus and inguinal nodes is not altered by estradiol. However, the increase in adrenal weight is somewhat greater in these animals. Leucocytes are decreased equally in both estrogen and control (sesame oil injected) irradiated animals, although maximal depression and recovery occur somewhat earlier in the former. Erythrocyte count of irradiated controls is decreased by 45% at 11 and 14 days after exposure. Estrogen treated animals manifest a smaller decrease in red count (25%) 7 days after irradiation and normal levels at 11 and 14 days. The possibility that the protective action of estrogens is mediated by the adrenals is being investigated.

Oxygen consumption and water balance in the X-irradiated frog. HARVEY M. PATT, MARGUERITE N. SWIFT (by invitation) and ELLA B. TYREE (by invitation). *Biology Division, Argonne National Lab., Chicago, Ill.* As part of a study of the radiation response of frogs and of factors contributing to their radiosensitivity, we have investigated: *1)* oxygen consumption after X-irradiation; and *2)* rate of water uptake in dehydrated irradiated frogs. Oxygen consumed by individual unfed Autumn frogs, sedated with nembutal, was measured volumetrically at atmospheric pressure and 23°C. Results of a pilot study in which determinations were made during a control period of several days and at frequent intervals after X-irradiation with 3000 R (completely lethal, mean survival time 26 days), suggest a slight early decrease in oxygen consumption with maximal depression (34%) 15 days after exposure. This is followed by return to control levels (0.086 ± .002 cc/gm/hr.) around 25 days after irradiation. Although there is evidence that ionizing radiation accelerates catabolism in mammals, especially terminally, we failed to observe any increase in oxygen consumption in these frogs. Body weight of unfed irradiated frogs also did not change appreciably. Frogs kept in dry individual containers at 23°C. for 3 days lose 35–40% of their water. After irradiation with 3000 R and immediate return to water, such partially dehydrated irradiated frogs regain water at the same rate as non-irradiated dehydrated controls. In both groups, recovery is essentially complete by 4 hours. There is no change in water content of non-dehydrated irradiated frogs over this period. Experiments are in progress to evaluate the influence of water content at the time of exposure on radiosensitivity in the frog.

Effect of urine extracts from hypophysectomized dogs on gastric secretion. T. L. PATTERSON, J. KAULBERSZ, D. J. SANDWEISS and H. C. SALTZSTEIN (by invitation). *Depts. of Physiology and Surgery, Wayne Univ. College of Medicine and Harper Hospital, Detroit, Mich.* In continuation of our earlier studies related to the influence on gastric secretion of urine extracts from dogs deprived of their pituitary gland, new series of experiments were made with extracts prepared from dogs hypophysectomized by transtemporal methods. The effect of the extracts were tested on several dogs either with gastric fistula or with Heidenhain pouch by the double-histamine technique. In 21 experiments (*series A*) the extract was administered during the second period of the double-histamine test, and in 25 experiments (*series B*) during the first period. Extracts from the urine of hypophysectomized dogs did not exhibit a prompt inhibitory effect which is characteristic for urogastrone prepared from normal urine. In relatively small doses (1 mg.) hypophysectomized extracts produced as an immediate effect an increase in secretory response to histamine which is statistically significant in both animal series. No significant delayed effect on secretion could be detected. In larger doses (3 mg. or more) there was no significant immediate effect on secretory response to

histamine, although in some dogs varied degrees of stimulation or inhibition were observed. However, statistically significant evidence of a strong delayed inhibition lasting more than 9 hours is revealed. Therefore, it appears that urine extracts prepared from hypophysectomized dogs contain both stimulatory and inhibitory agents. The stimulatory effect is prompt and more noticeable in small doses. The inhibitory effect is more evident in larger doses of the extract. In contrast to that of typical urogastrone, inhibition develops slowly and lasts longer than 9 hours.

Manifestations of potassium intoxication in the dog heart-lung preparation. FRANCIS E. PAYNE (by invitation), LESLIE L. BENNETT, SANDOR G. BURSTEIN (by invitation), and JAMES HOPPER, JR. (by invitation). *Divisions of Physiology and Medicine, Univ. of California, Berkeley and San Francisco, Calif.* Heart-lung preparations were arranged for the continuous recording of right auricular pressure, aortic pressure, ventricular volume, and left ventricular output minus the coronary flow. Unipolar electrocardiographic tracings were taken directly from the anterior surface of the left ventricle using a wick electrode. The plasma potassium concentration was elevated by the addition of isotonic KCL and the plasma level was determined by the use of a flame photometer. Eight heart-lung preparations were studied. The venous return and peripheral resistance were not altered during an experiment. It was found that the major mechanical phenomena of potassium intoxication did not appear until a plasma level of approximately 7mEq/l. was reached. While approaching this level of potassium concentration only minimal increases in ventricular volume and minimal decreases in cardiac rate and output were observed. During this period of increasing potassium concentration there were progressive electrocardiographic changes which consisted of decreased amplitude of P waves and QRS complexes, depression of the S–T segments, prolongation of the P–R intervals, and increased amplitude of the T waves. When potassium levels of approximately 7mEq/l. were reached the following marked changes of abrupt onset occurred: increase in right auricular pressure, ventricular volume, and aortic pulse pressure (reflecting the increased stroke output due to the slow rate); and decrease in cardiac output and heart rate (the result of auricular standstill). The most striking associated electrocardiographic changes were the disappearance of the P waves, further displacement of the S–T segments and still higher T waves.

Hepato-renal factors in circulatory homeostasis. XXV. Low protein diets and renal VEM formation. MARY ANN PAYNE (by invitation) and EPHRAIM SHORR. *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* Previous studies from this laboratory demonstrated the importance of the renal vasoexcitor, VEM, for compensatory peripheral vascular reactions to hemorrhagic and traumatic shock; in its absence, these compensatory reactions failed to occur. Under certain conditions the renal capacity to form VEM was abolished; e.g., anoxia (2 hours for rat, 4 for dog kidney), adrenal insufficiency and nutritional cirrhosis (rats, on 10% protein diets for 4 months). The present study deals with the acute effects, in rats, of fasting and a lower protein diet (5% casein) on the renal capacity to form VEM, as measured by anaerobic incubation of kidney slices *in vitro*. Adequate vitamin supplements were supplied throughout. Fasting rats sustained a significant reduction in the capacity to form VEM by the 4th, and a total loss by the 6th day of the fast. Those receiving the 5% casein diet showed appreciable reductions in VEM formation by the 8th, and complete abolition from the 12th through the 39th day, when the experiment was terminated. Desoxycorticosterone acetate, which restores VEM formation to kidneys of adrenalectomized rats, failed to prevent this defect, when given from day 1 to 14, or to correct it, when given from day 14 to 39 of the low protein regime. This VEM defect was not associated with significant reductions in renal oxygen consumption. It is suggested that, in addition to the adrenal cortical hormones and an aerobic environment, an adequate protein intake is essential for the integrity of the renal mechanism for forming VEM.

Studies on the fat-mobilizing hormone of the anterior pituitary gland. RICHARD W. PAYNE (introduced by E. B. ASTWOOD). *Joseph H. Pratt Diagnostic Hospital, and Dept. of Medicine, Tufts Medical School, Boston, Mass.* The fat-mobilizing effect of various anterior pituitary preparations was assayed by fat deposition in the livers of fasted mice by the method of Campbell (*Endocrinology* 23: 692, 1938). Increasing doses induced a progressive increase in the liver fat from control values of 4.5 to 6.0% to maximal values of 12.0 to 14.0%. Higher doses caused a submaximal response. Some activity was found to be present in all anterior pituitary preparations tested (whole beef anterior pituitary and pork and sheep pituitary powders, Armour and Parke-Davis thyrotrophic hormone, Armour's adrenocorticotrophic hormone, Armour's growth hormone, and Searle's anterior pituitary gonadotrophic hormone). Posterior pituitary preparations showed no fat-mobilizing properties. Purified adrenocorticotrophic and thyrotrophic hormones were the most active on a weight basis. Purified growth hormone and gonadotrophic hormones showed little activity as compared to other purified preparations. One week after adrenalectomy, the test animals exhibited no fat-mobilization in response to any anterior pituitary preparation. Thyroidectomy or depression of thy-

roid function with propylthiouracil did not inhibit the effect. Single injections in oil of progesterone (10 mg.), testosterone (25 mg.), desoxycorticosterone acetate (5 mg.), and stilbestrol (5 mg.) had no effect. One cc. of aqueous adrenal cortical extract produced no significant fat-mobilizing effect.

Comparison of a volumetric respirometer with the Warburg method in measurement of brain respiration. C. N. PEISS and R. WENNESLAND (introduced by J. FIELD). *O.N.R. Arctic Research Laby., Point Barrow, Alaska, and Dept. of Physiology, Stanford Univ., Stanford, Calif.* Six aliquants (each about 25 mg. wet weight) of rat cerebral cortex slices were prepared from the brains of each of 8 adult albino rats. The moist cold box technique was used (Field, *Methods in Medical Research*, 1948). Oxygen uptake was measured on 3 aliquants by the Warburg method and on the other 3 by means of volumetric respirometers (see abstract by Wennesland). Thus 48 Warburg runs were made and 47 volumetric (one sample lost). The liquid phase was Krebs Ringer's phosphate containing 0.011M glucose and 0.01M bicarbonate. Gas phase oxygen. Temperature 37.5 ± 0.01°C. Initial *p*H 7.4. Final *p*H 7.4 to 7.5. The mean Q_{O_2} s were 14.12 and 14.08 for the Warburg and volumetric methods respectively. The corresponding standard errors were ±0.169 and ±0.171. Statistical analysis showed that the two series did not differ significantly either in respect of the means (Student's 't' test) or of the variability (Fisher's 'F' test).

Blood gas content of dogs during immersion hypothermia. K. E. PENROD and FELIX R. ROSENHAIN (by invitation). *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Simultaneously drawn samples of arterial blood from the carotid artery and mixed venous blood from the right ventricle were handled anaerobically and analyzed for O_2 and CO_2 content. Samples were drawn before immersion in an iced bath and at rectal temperatures of 30°, 25° and 20°C. while breathing air; and before immersion, at 35°, 30° and 25°C. in a second series breathing 100% O_2. All dogs were lightly anesthetized with sodium pentothal. Hematocrit determinations were made on each blood sample. In 2 of 9 dogs breathing air a mounting arterial CO_2, evidence of approaching respiratory inadequacy, appeared above 25°C. rectal temperature. In 4 it was between 25° and 20° and in the other 3 below 20°C. Beginning circulatory inadequacy was not yet evident at 20°C. rectal temperature in 7 of 9 dogs. In one it occurred between 25° and 20° and in one failure was progressive during the entire period of immersion. In 5 of 8 dogs surviving to below 20°C. the venous O_2 content at 20° was equal to or greater than pre-immersion levels. Hematocrits showed a 20% increase during hypothermia which is partially accounted for by pentothal depression of the control value. When breathing 100% O_2 throughout the hypothermic period 4 of 9 dogs showed some respiratory failure above 25°C. In nearly every case respiration was depressed throughout, as reflected in a high arterial CO_2 content. Circulatory impairment was evident in only 1 dog at 25°C. The venous O_2 content was higher at 25°C. in 7 of the 9 dogs than it was before immersion.

Cooling as a stimulus to certain smooth muscles. JOHN F. PERKINS, JR. and CHARLES H. NICHOLAS (introduced by R. W. GERARD). *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* Cooling alone causes contraction of the retractor penis muscle of the dog (De Zilwa, *J. Physiol.* 27: 200, 1901), of the cat's nictitating membrane (Perkins and Li, *Fed. Proc.* 7: 1948) and apparently also of the smooth muscle of the arteriovenous anastomoses of the skin (Perkins et al., *Am. J. Physiol.* in press). Further studies were made on the dog's retractor penis, which was placed in Tyrode's solution in a jacketted bath. Temperature was measured by a thermistor in contact with the muscle. Provision was made to cool the muscle gradually, or rapidly, from 36°C. down to as low as 0°C. Cooling alone acts as a stimulus, producing as quick a contraction as that caused by epinephrine. Rapid cooling is a more effective stimulus than gradual. When degrees of rapid cooling (abcissae) were plotted against heights of contraction, an S-shaped curve resulted, with a maximum at 24° of rapid cooling (cooling from 36 to 12°C.). Lowering the *p*H to near 7 causes a gradual, marked contraction, on which the effect of cold can be superimposed. The separate effects of epinephrine and of cooling are additive when tested together. After Dibenamine the muscle lengthens with epinephrine but still shortens with cold. With both together, the effects tend to cancel (algebraic addition). Thus, cold apparently acts on a different excitatory system than epinephrine, or else it acts directly on the contractile system. Against a direct action is the failure of rabbit intestine, uterus, aorta, and iris to contract when cooled.

Effect of estrogen and relaxin on the connective tissue of the pubic symphysis. E. PERL (by invitation) and H. R. CATCHPOLE. *Dept. of Pathology, College of Medicine, Univ. of Illinois, Chicago, Ill.* Female guinea pigs castrated for 1 month were treated daily for 4–5 days with 1.0 μg estradiol, then given an injection of pregnant rabbit serum containing relaxin. Following relaxation (6–8 hours) each of one group of animals received intravenously 4.0 ml 1.25% Evans blue (intravital) dye, and was killed 10 minutes later. The symphysis was frozen in liquid air and frozen-dried *in vacuo*. The dried block was embedded in paraffin and sectioned at 120 mμ. Uninjected controls and animals receiving estrogen only were similarly studied. In another group, symphyses of control, estrogen treated and

estrogen-relaxin treated animals were frozen-dried, embedded as above, sectioned at 5–10 mμ and stained for glycoproteins by the McManus-Hotchkiss periodic acid-leucofuchsin reagent. Connective tissue between the symphyseal cartilages is unstained by Evans blue in control, and virtually unstained in estrogen treated, animals; in the anterior and posterior notches bordering the symphysis, estrogen treated animals show slight staining. Following relaxin the connective tissue of the joint proper stains more or less blue in all cases and that of the notches stains very markedly. The glycoprotein material of the symphysis, which stains with periodic acid-leucofuchsin reagent, was found to become more soluble in aqueous solvents following relaxin. The interpretation is made that relaxin induces breakdown (depolymerization) of the glycoproteins of the symphyseal region, which provides the physical basis for relaxation; these components become more water soluble and are able to bind Evans blue.

Mixing time of Brilliant Vital Red injected into dogs. RAYMOND W. PICKERING (by invitation) and PHILIP DOW. *Dept. of Physiology, Univ. of Georgia School of Medicine, Augusta, Ga.* The mixing time of intravascular dyes injected into normal animals has long been controversial. Its prolongation in circulatory collapse has been recognized but not (so far as we have found) quantitatively measured. An opportunity to obtain estimates of both was presented in the course of efforts to follow fluid shifts during serial hemorrhages in dogs (currently reported by Remington). Following a rapid jugular injection of Brilliant Vital Red, samples at frequent intervals were drawn simultaneously from a femoral artery and the corresponding vein. Under control conditions, arterial dye concentrations declined and venous concentrations rose, the two becoming acceptably identical after four minutes. After an estimated 35% hemorrhage (7 steps, 1 hour) the results were highly variable. In one type the concentration curves were like controls in form but greatly prolonged, their slow approximation permitting an estimate of dilution volume only after 20 to 30 minutes. In another type both arterial and venous concentrations continued to rise for 30 minutes, suggesting sequestration elsewhere and slow mixing of a large fraction of the injected dye. When supplementary samples from the non-injected jugular were added to the series they coincided with the arterial curve and would thus have given false assurance of mixing had they been the only ones available for comparison. The results emphasize the hazardous aspect of estimating completeness of mixing from a series of samples from any single vessel. They supplement and extend the previous observations of the Gregersen group and of Phillips.

Potent pressor action of an extract of a phaeochromocytoma after adrenolytic doses of Dibenamine. D. M. PITCAIRN (by invitation) and W. B. YOUMANS. *Dept. of Physiology, Univ. of Oregon Medical School, Portland, Ore.* A 13-year-old boy with severe and relatively sustained arterial hypertension was given Dibenamine, and the blood pressure decreased rapidly to the normal range with the patient remaining in the supine position. Following surgical removal of the left adrenal gland, which contained a phaeochromocytoma, his hypertension was relieved. An aqueous extract of the phaeochromocytoma was prepared 2 hours after removal, and effects of the extract upon the blood pressure of dogs under pentobarbital sodium were determined before and after Dibenamine. The extract had a very potent pressor action which was not blocked by full adrenolytic doses of Dibenamine. The pressor substance was present in slightly diminished amount in the refrigerated phaeochromocytoma 24 hours later, and a relatively small amount was detected after 5 days. Extracts of the thin layer of cortex, which could not be cleanly separated from the phaeochromocytoma, had much less pressor potency than extracts from the central portion of the tumor. Aqueous extracts of fresh normal dog and beef adrenal medulla, like epinephrine, had pressor action before Dibenamine and depressor action after Dibenamine. Aqueous extracts of dog and beef adrenal cortex produced either a slight fall in blood pressure or no response following Dibenamine. Persistence of the major part of the pressor action of the extract of the phaeochromocytoma after Dibenamine indicates the presence in the tumor of a considerable amount of a pressor substance other than epinephrine.

Filtration rate and sodium and water excretion following mercurial diuretics. ROBERT F. PITTS and JOHN J. DUGGAN (by invitation). *Dept. of Physiology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* The rate of elimination of sodium and water by the normal human subject following the intravenous administration of 2 cc. of mercuhydrin is determined in part by the glomerular filtration rate. Two subjects hydrated with 500 cc. of water per os prior to the start of the experiment showed evidence of mild to severe peripheral circulatory failure from 1.5 to 2.5 hrs. following the mercurial. Glomerular filtration rate was depressed during the period of circulatory collapse, as was the rate of excretion of sodium and water. When the subjects were hydrated by the infusion of saline at 10 to 15 cc. per min. for 2 hr. prior to the start and throughout the course of the experiment, glomerular filtration was maintained at a normal or elevated rate for the subsequent 5-hr. period of observation. The quantities of salt and water eliminated in the latter experiments far exceeded those of the preceding ones. Salt and water balances and hematocrit changes suggest that diuresis in the

water hydrated subjects rapidly reduced circulating blood volume and led to circulatory collapse with its attendant fall in filtration rate. A greater fluid reserve in the saline hydrated subjects, prevented these changes. It is inferred that a decrease in filtration rate is attended by reduced delivery of proximal tubular fluid into the distal segment, the sodium and water absorptive capacity of which is depressed by mercurial diuretics. Accordingly little sodium and water may be excreted even though distal absorption is severely curtailed.

Macrophage promoting factor (MPF) in mammalian blood serum. C. M. POMERAT and M. F. ORR (by invitation). *Tissue Culture Laby. Medical Branch, Univ. of Texas, Galveston, Texas.* Fragments of spleens from 18-day chick embryos approximately 1 mm. square show a circular zone of migrating cells about 5 mm. in diameter within 24 hours when cultivated at 37°C. in a clot formed of cockrel plasma and chick embryo extract. The peripheral elements of such cultures consist of myelocytes with acidophilic granules. While the inclusion of 25% mammalian serum in the composition of the medium gave typical cultures, it was found that the blood of some rabbits induced the appearance of large numbers of highly phagocytic cells which engulfed all outwandering myelocytes within 24 hours. This macrophage promoting factor (MPF) has been found to be thermolabile, precipitated by 1/3 saturated ammonium sulfate and to be present in an acetone insoluble fraction. It does not appear to be related to the Forssman antigen or to anti-organ sera with high or low complement-fixation or hemolytic titres. It was not found possible to reproduce the phenomenon of macrophage induction under the conditions described with the use of various concentrations of histamine, 17-hydroxy-11-dehydrocorticosterone, choline or various quaternary ammonium compounds. MPF does not appear to be identical with leucotaxin, the leucocyte-promoting factor, necrosin, pyrexin or the leucopenic factor which have been found by Menkin in experimental inflammatory exudates.

Estimate of the kinetic and potential energy of the work of the human heart. O. PREC (by invitation), L. N. KATZ, R. ROSENMAN (by invitation) and L. W. SENNETT (by invitation). *Cardiovascular Dept., Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* The recent techniques of angiocardiography and right heart catheterization offer data from which an approximate calculation of the work of the human heart may be determined from the formula: $W = \left(PV + \frac{Mv^2}{2g}\right)_L + \left(PV + \frac{Mv^2}{2g}\right)_R$. The significance of the various symbols are those currently accepted. V and M are calculated from the Fick formula; v is calculated from the stroke output, cross sectional area of the aorta, just above the sinuses of Valsalva, and of the pulmonary artery, and the ejection time. The last is obtained from the pressure pulse within the main pulmonary artery. Data on a typical case are as follows: aortic diameter, 3.10 cm.; pulmonary artery diameter, 3.23 cm.; stroke output, 104 cc.; ejection period, 0.26 sec.; mean aortic pressure (estimated), 135 mm. Hg; mean pulmonary arterial pressure, 14 mm. Hg. Work in gm.cm/sec. = 18,250 + 160 + 1900 + 130 = 20,440. Kinetic energy equals 6.8% of the total work of the right ventricle, and 0.9% of the left ventricle. The kinetic energy equals 1.4% of the total work of both ventricles.

Studies on the accuracy of pressures recorded through a catheter. W. H. PRITCHARD (by invitation), R. W. ECKSTEIN, R. ECKEL, (by invitation), C. L. PARSONS (by invitation) and T. E. LOWE (by invitation). *Western Reserve Univ., Dept. of Medicine, Cleveland, Ohio.* The wide use of the catheter for pressure recording demands a critical comparison of its recorded curves with those recorded by more adequate means. For these studies, pressures were recorded through catheters connected to a high frequency Gregg optical manometer (with and without various lengths of intervening lead tube). These were compared to pressures simultaneously recorded through large needles connected by short lengths of lead to a second Gregg optical manometer. Pressures were measured in a diaphragm type of pump, and the right ventricle of the anesthetized open chest dog. With the pump perfect pressure agreement occurred at slow pump speeds. Higher speeds and the addition of lead tube produced artefacts with marked overshooting and unreadable curves. Time lags were small, from 0/008 to 0/015 sec. In the dog the catheter also produced overshooting with secondary oscillations making true pressure estimations impossible. This inadequacy was accentuated by the addition of 1 or 2 meter lengths of lead tube. The curves were markedly improved when a certain degree of clotting occurred in the catheter tip. Therefore, damping was done by partially closing a stopcock placed between the catheter and manometer. At damping coefficients of about .7 accurate readable curves were obtained. Readable curves were also obtained from the human right ventricle after ideal damping with a stopcock.

Heat loss and heat production of cats at different environmental temperatures. LAWRENCE R. PROUTY (introduced by EUGENE F. DU BOIS). *Dept. of Physiology, Cornell Univ. Medical College, New York City.* Shaved, unanesthesitized cats were studied during 77 one-half hour periods in environments ranging from 19° to 39°C. have shown that the thermoregulatory mechanisms of a cat differ greatly from man and that the cat is a poor laboratory subject for the study of human temperature regulation. Simultaneous determinations of respiratory metabolism, heat loss, rectal and average skin

temperatures and thermal conductance were made in the gradient calorimeter previously described (Prouty, Barrett and Hardy: *Fed. Proc.* 7: 96, 1948). Relative humidity was kept low enough not to interfere with the efficiency of the evaporative process. As environmental temperatures were decreased below thermal neutrality, heat loss became progressively greater than heat production. In 20 experiments between 20° and 24°C. heat loss was 16% greater. Vaporization values of 9 to 15 gm/m²/hr. were approximately the same in this range as in the zone of thermal neutrality. Man can alter peripheral thermal conductance 5–6 fold but the cat shows a relatively flat conductance curve until it licks itself. In the cold, the cat relies upon the protection of its fur and the ability to decrease its surface area or increase its heat production. Without fur, rectal temperature drops to 35°C. then falls precipitously and death would result if the animal could not find a warmer climate. As environmental temperature is increased above thermal neutrality, heat production becomes increasingly greater than heat loss. At 38.5°C., it is 75% greater. At 39°C., respiration approximates 200/min. after 2 hours. There is a rapid rise in rectal temperature to 43.9 and skin temperature to 43.5°C. The animal does not sweat appreciably but coats accessible parts with saliva. Nausea, vomiting and convulsions occur and the animal must be removed quickly.

Effect of protein in the diet upon certain aspects of renal function. THEODORE N. PULLMAN (by invitation), ALF S. ALVING, and MILTON LANDOWNE. *Dept. of Medicine, Univ. of Chicago, Chicago, Ill.* Ten normal young adults were placed on each of 3 diets, containing different amounts of protein, for successive 2-week periods. The low, intermediary, and high protein regimens contained respectively 0.3–0.4, 1.0–1.1, and 2.3–3.0 gm. of protein/kg. of body weight/day. Simultaneous inulin and diodrast clearances were determined after 2 weeks on each diet. Eight subjects showed higher glomerular filtration rates on the high than on the medium protein diet, the total average increase being 21.9%. This difference was highly significant statistically. Six subjects showed lower glomerular filtration rates on the low protein than on the control diet, but the average difference could not be proved significant. Seven individuals showed a higher renal plasma flow on the high than on the medium protein diet, the total average increase being 20.0%. This difference was also statistically of high significance. Eight subjects had lower renal plasma flows on the low than on the control diet, but the average difference could not be proved significant. No significant change in filtration fraction occurred. Preliminary studies on 6 subjects indicate that urea clearances are lower on the low protein diets and that such changes cannot be accounted for solely by diminution in the glomerular filtration rate. In all groups, the tubular reabsorption rate of urea seemed related linearly to the filtration rate of urea, the slope being higher when dietary protein was low than when medium or high. This relation is currently under further investigation.

Effects of unequal blood flow and ventilation upon the alveolar-arterial gradient. HERMANN RAHN. *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* It has previously been shown that, given a mixed venous blood gas content and inspired air tension, all the possible simultaneous combinations of pO_2 and pCO_2 existing in the lung or parts thereof can be determined. If the metabolic R.Q. is known then in addition the mean alveolar air composition can be defined. Any particular alveolar gas tension is determined by a definite ratio of alveolar ventilation (V) to pulmonary blood flow (F). When both of these quantities are measured in the same units then

$$\frac{V}{F} = \frac{.864 \times (A\text{-}V)\ O_2 \text{ difference} \times R.Q.}{\text{alv. } pCO_2}$$

Thus a mean alveolar gas composition is determined by a definite mean V/F ratio. But to calculate the mean arterial pressures one needs to know not only the alveolar pO_2 and CO_2 produced by the mean V/F but also by the variance of V/F. This variance is determined by the degree of unequal pulmonary perfusion and alveolar ventilation. While the gradient for CO_2 is neglible, the O_2 gradient depends upon the magnitude of the deviations assumed.

Relations between blood flow, skin temperature, evaporation rates and sweating in various skin regions. WALTER C. RANDALL and ALRICK B. HERTZMAN. *Dept. of Physiology, St. Louis Univ. School of Medicine.* Simultaneous measurements of cutaneous blood flow, evaporation rates, and surface temperatures following mecholyl iontophoresis suggested certain important correlations between these functions in maintaining thermal flows at the skin surface. In these experiments involving high sweating rates, the skin may be partitioned into three zones: *1*) areas such as the dorsal surfaces of the hand and foot where sweating rates provide for an evaporative heat loss in excess of the heat delivered by the blood; *2*) surfaces of the face where sweating rates are low with relatively high blood flows; *3*) areas of the trunk where both maximal blood flows and sweating rates are intermediate between these extremes. The largest total surface area falls within the latter group. Calculations of total possible evaporative heat losses if all the sweat were vaporized from each of these areas suggest a marked initial thermal deficit on the part of the cutaneous blood flow. Part of this deficit may be corrected by direct conduction from deeper tissues. Such thermal deficits have been observed immediately following stimulation of the sweat glands and blood vessels by mecholyl, but the deficit rapidly de-

creases to a point where appropriate caloric balances may be established.

IV. An electronic, multi-range, multi-channel, direct-writing pressure recorder. MAURICE RAPPAPORT and STANLEY J. SARNOFF (introduced by WILLIAM H. FORBES). *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.* An apparatus has been developed which combines the characteristics thought to be desirable in a pressure recording apparatus. *1*) Linearity over all pressure ranges. *2*) Small fluid shift with change in pressure (0.02 mm.^{3} for 100 mm. Hg). Change of sensitivity without changing the natural frequency of the system thus enabling full-scale deflection for 400 mm. Hg, or for 1 mm. Hg pressure change (with appropriate intermediate sensitivities) by simple adjustment of the amplification circuit. *3*) Recording by means of a direct-writing galvanometer. *4*) Rectilinear rather than curvilinear coordinates. *5*) Multi-channel recording. *6*) Portability of the single channel apparatus. 7) Multiple chart speeds ranging from 0.25 mm. to 50 mm. per second with intermediate speeds. *8*) Sterilizability of all components in contact with the fluid system. *9*) Additional recording channels for synchronization with the electrocardiogram, pneumotachogram, pneumogram, and phonocardiogram, electrokymogram, etc. The pressure pick-up is a condenser microphone which is an element of an alternating current bridge, operating at radio frequencies. When zero pressure is applied to the pressure pick-up, the bridge is balanced and the output from it is zero. When pressure is applied, the bridge is proportionately unbalanced, and a voltage output therefore occurs across the bridge. This voltage is amplified and rectified and fed into a direct writing or photographic galvanometer.

Multiple avitaminosis-like syndrome produced by adenine. SIGWIN B. RASKA. *Dept. of Pharmacology, Univ. of Mississippi School of Medicine, University, Miss.* Orally administered adenine in daily doses of 30 to 50 mg/kg. given for a period of about 3 to 5 weeks produces the syndrome of multiple avitaminosis in dogs receiving complete normal diets both before and during the experimental period (Raska, *Science* 105: 1947; *J. Biol. Chem.* 165: 743, 1946). Gross and microscopic examination of the tissues of these animals disclosed the following: scattered areas of atrophy of the epithelium of the inner surface of the lip with dense cellular infiltration in the underlying tissue; general atrophy of the buccal mucous membrane with erosion in the depth of the buccal folds and moderate inflammatory leucocytic infiltration in the subepithelial tissues; varying grades of necrosis of the tongue, from moderately severe to marked, characterized by extensive pyknosis and even by obliteration of cross-striations and fibrils as the consequence of coagulation necrosis of the muscle fibers; extensive necrosis of the gastric mucosa extending as deeply as the muscu'aris and accompanied by considerable hemorrhage in the necrotic tissue; deposition of brownish crystalline material in the distal convoluted tubules and collecting tubules of the kidney and monocytic infiltration in the interstitial tissue; deposition of calcareous material in some tubules; granulomatous lesions scattered throughout the renal cortex; moderate congestion of the pancreas; considerable congestion of the adrenal medulla and the liver. In these animals, blood NPN, blood urea and blood uric acid were markedly elevated. Oliguria and abnormal urinary findings (e.g., large numbers of WBC, hyaline casts and large amounts of amorphous sediment) were present. In the early stages, arterial blood pressure was moderately but definitely increased. The motor activity of the animals indicated pathologic changes in the central nervous system. Vitamins of the B complex were able to counteract partially the effects of adenine only in the early stages of the syndrome.

Age and kidney renin in rabbits. G. CARL RAU (introduced by M. M. ELLIS). *Dept. of Physiology and Pharmacology, Univ. of Missouri Medical School, Columbia, Mo.* Grossman and Williams (1938) reported that kidneys of young rats contained more renin than those of older rats, using extracts which were not hypertensinase-free and may have contained other pressor substances, for Helmer and Shipley (1947) suggest that kidney contains another pressor principle distinct from but extractable with renin. It seemed desirable to determine whether the amount of renin alone in the kidney varies with age, using extracts prepared by newer methods and known to be free from other pressor substances and hypertensinase. With modifications the preparation of renin, hypertensinogen and hypertensin, and the assay procedures were according to Dexter, Haynes and Bridges (1945). Sixty rabbits of three age groups, 20–24, 180 and 365 days old, were used, i.e., two groups of immature and one of adult rabbits. Each group of 20 was divided into two lots of 10 and the renin extracted from the pooled kidney masses of each lot. On the basis of gram of kidney, considering the 365-day group as 1, the renin yield for the 180-day group was 5 and for the 20 to 24-day group, 2. When the proportional kidney weight was used, i.e. kidney weight divided by body weight, the renin yield per gram of kidney, considering the 365-day group 1, was 5 for both the 180 and the 20 to 24-day group. The renin content of kidneys of immature rabbits as found was definitely greater than that of adult rabbits.

Effect of position upon the respiratory rate of anesthetized dogs. E. A. REED and J. C. SCOTT. *Dept. of Physiology, Hahnemann Medical College, Philadelphia, Penna.* Respiration was recorded by

a tambour and endotracheal tube in dogs anesthetized with nembutal. Oxygen was supplied by a second endotracheal catheter. Compared with the lateral, the supine position usually increases the rate while the prone markedly decreases it. The rate is greatly increased in the supine position by tilting head downward 27°. Tilting head upward 27° decreases the rate. The head upward and the prone positions may completely inhibit respiration for several minutes. A return toward the rate of the previous position occurs during the first few minutes. When the change of position is one producing acceleration, the increased rate during the first minute may be due partly to extraneous stimulation from handling the animal. However, when the change of position produces a decreased rate, the same apparent adaptation proceeds in the reverse direction. Bilateral cervical vagotomy greatly reduces or abolishes rate changes due to position. In many cases, the effect of the prone and the head downward positions is reversed. In most instances, there is an acceleration lasting for approximately one minute after change to any position. Rate changes in the intact animal are probably due mainly to the Herring-Breuer reflex. The craniventral slope of the diaphragm would permit compression of lung tissue by gravity shift of abdominal viscera when the animal is in the supine position and distension when in the prone position. Elevation or depression of the head end of the animal also distends or compresses lung tissue by shift of abdominal viscera.

Reduction of the plasma potassium concentration by vivodialysis and its restoration in non-visceral regions. ROGER M. REINECKE and FRANCIS L. STUTZMAN (by invitation). *Dept. of Physiology, Univ. of Minnesota, Minneapolis, Minn.* The arterial plasma potassium level of the dog was substantially reduced by vivodialysis. Concurrently there was a definite arterio-venous increase in the plasma potassium concentration of blood flowing through the hind limb. This arterio-venous increase was also found for blood flowing through a muscle that had been isolated from the body except for its blood and nerve supply.

Hemodynamic changes in response to hemorrhage with and without Dibenamine. JOHN W. REMINGTON. *Dept. of Physiology, Univ. of Georgia School of Medicine, Augusta, Ga.* Circulatory changes accompanying serial hemorrhages have been followed in 20 control dogs, and in 16 animals given Dibenamine. On the average, the first of the 6 or 7 bleedings (5 cc,kg. each, at 10-min. intervals), was followed by a 7% fall in mean blood pressure (optical manometer) and a 26% reduction in flow (calculated from pressure pulse contour), indicating 18% increase in resistance. Mean pressure was then held almost constant through the 4th hemorrhage, while flow declined slowly to —39%, and resistance increased to +30%. Cardio-acceleration started usually after the 2nd hemorrhage, reaching its maximum after the 5th. In the late hemorrhages, when pulse rate was nearly maximal, mean pressure declined rapidly, with a reduction in resistance to pre-hemorrhage levels. The changes in the above factors were parallel in the two groups, which did not differ significantly. During the cardio-acceleration, the fracion of total blood volume held in the heart and lungs (measured by the dye injection technique) was reduced. X-ray heart volume and electrocardiographic changes in the two series are being studied. There was little if any dilution of the plasma (falling drop) until after the 4th hemorrhage, when dilution began at a rapid rate. The causal basis of the time of onset and the degree of dilution are not clear. Central venous pressure declined during the early hemorrhage, but later changes were variable.

Analysis of out-of-doors thermal stress due to radiation with the pan-radiometer. CHARLES H. RICHARDS (by invitation) and JAMES D. HARDY. *New York Hospital and the Dept. of Physiology, Cornell Univ. Medical College, New York City.* The fact that the solar spectrum and the emission spectrum of the low temperature radiation of the environment do not overlap appreciably makes it possible to measure separately these sources of radiant energy with the pan-radiometer. This instrument now consists of 3 hollow silver spheres, each containing a heating coil and thermocouple. The outer surface of one sphere is highly polished, a second is dull black, and the third is matte white reflecting the sun's radiation. When this instrument is exposed out of doors in sunlight, the black sphere becomes warmest, the polished sphere next and the white sphere least. Heat is put into the white and polished spheres bringing them to the temperature of the black sphere. From a measurement of this heat and the black sphere temperature the following can be calculated: the direct radiant heat of sun, the albedo of the environment, and the equivalent radiant temperature of the environment. These data, combined properly with the air temperature and velocity, will give the operative temperature which is a measure of the thermal stress of the environment.

Viscosity of muscle protoplasm. PETER RIESER (introduced by L. V. HEILBRUNN). *Zoological Laby., Univ. of Pennsylvania, Philadelphia, Penna.* Small oil drops were microinjected into muscle fibers from the adductor magnus of the frog, and the rise of the drops through the protoplasm was observed in a horizontal microscope. The protoplasmic viscosity was determined from Stokes' law. An average value of 29 centipoises was obtained. Some higher values were interpreted as representing cases where a slight degree of injury had occurred. Visible clots always caused stoppage of the

movement of the drops. Oil drops were able to move only in one direction through the fibers. No movement across the fibers was ever observed. Moreover, no movement of the drops could be noted in moribund fibers in which longitudinal fibrillar elements had become visible. These facts, as well as the ovoid shape of the drops, suggest the existence of some longitudinal structural arrangement within the fibers. Microinjection of long stretches of fibers with oil or air always showed that a peripheral region directly within the sarcolemma could never be displaced by these substances. This outer region had a thickness of approximately 10μ. By microinjecting aqueous solutions it was possible to push the protoplasm ahead through the fibers. In every instance where such a flow occurred, there was a peripheral region of approximately the same thickness as noted above, and this peripheral region did not flow. The outer region thus appears to be similar to the gel-like cortex of other types of cells.

Analysis of ventilation-perfusion relationships in the lungs. R. L. RILEY and A. COURNAND. *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, New York City.* The composition of alveolar air and of the blood leaving the alveolar capillaries in any part of the lung depends upon the ventilation-perfusion ratio, and conversely, analysis of the ventilation-perfusion ratio requires that the composition of the alveolar air be known. Since the alveolar air differs in different parts of the lungs, especially in diseased states, calculations are facilitated by the use of an 'ideal' value, defined as the one and only value for the composition of alveolar air which satisfies both the blood R.Q. and gas R.Q. equations, assuming perfect equilibrium between the blood and gas leaving each alveolus. This is the only value for alveolar air which could exist homogeneously throughout all functioning alveoli and still be compatible with the quantitative aspects of gas exchange which actually exist in a given subject. The concept of 'ideal' alveolar air makes possible the calculation of a value for 'dead space' which includes a contribution from alveoli which are well ventilated but poorly perfused (high Va/P ratio). It also makes possible the calculation of a value for 'venous admixture' which includes a contribution from alveoli which are well perfused but poorly ventilated (low Va/P ratio). These relationships have been studied quantitatively in normal individuals and in patients with chronic pulmonary disease.

Measurements of heart output with the electrokymograph. GORDON C. RING, M. J. OPPENHEIMER and MIRIAM BALABAN (by invitation). *Temple Univ. Medical School, Philadelphia, Penna.* Measurement of the stroke volume of the heart by means of the roentgenkymograph (*Am. J. Physiol.* 126: 741, 1939) has never been widely used probably because the heart borders are so poorly defined. The use of x-rays with the electrokymograph (EKY) appears to offer a more satisfactory method because the movements of the heart borders can be amplified and the records very accurately measured. To test whether such records are suitable for calculating heart output, we have compared the results using our method with those obtained from ballistocardiograms (*J. Clin. Invest.* 26: 1, 1947) simultaneously recorded. It was found that the measurement of heart output by means of the E.K.Y. gives results comparable with those obtained by the ballistocardiograph. Only 5 of 30 records on normal medical students and nurses showed differences between the two methods of more than 20%. Since the cardiac output measured by ballistic methods checks the Fick no closer than this, our procedure may be as good as or better than the ballistocardiograph. It also provides other valuable information. With the subject lying on a ballistocardiograph table, the head of the electrokymograph is so placed that its fluorescent screen which is 3 cm in diameter is judged to be over the middle of the ventricular x-ray shadow. Changes in intensity of the fluorescent light are signaled by a current from a 931 A tube which activates a string galvanometer. These records are calibrated by passing a piece of pressed wood into and out of the x-ray beam. The formula used in the calculations is: Stroke = 10.55 (T^2 diast. — T^2 syst.) where T is the dorso-ventral thickness in centimeters.

Electrograms of contracting actomyosin threads. JANE SANDS ROBB and SAMUEL MALLOV. *Dept. of Pharmacology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* Electrodes penetrate a drop of solution into which threads of actomyosin are drawn. Connections by way of Offner amplifiers allow records to be made either with an ink writer, a research electrocardiograph or a Dumont oscilloscope. During control procedures many possible sources of extraneous currents were investigated, eliminated whenever possible, and at least are recognized when unavoidably present. Under controlled conditions potentials appearing when ATP is added to salt solution die away quickly leaving a steady base line. If ATP is made to disintegrate (heat or acid) potentials last far longer. No potential waves are recorded from a quiescent actomyosin thread. When a thread is activated potentials do appear which continue until full contraction is reached, after which time the base line is again steady. If another thread is drawn into, the same drop contraction and further potential differences occur. The intervening steady base line favors a theory associating these potential changes with thread activity. The form of the recorded waves varies with the setting of the instrument. Multiple channels allow simultaneous recording with varied settings. If high frequencies are favored bursts of fine peaks appear. If the setting is as for electro-

cardiography, the characteristic record consists of a series of slow monophasic waves each of which seems to precede a local contraction. Amplitude varies with the nearness and orientation of the thread to electrodes but less obviously to thread diameter.

Effect of some xanthine derivatives on gastric secretion. C. R. ROBERTSON (by invitation) and A. C. IVY. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Some xanthine derivatives have been reported to show an anti-histamine effect on smooth muscle in the cat. This led us to investigate their effects on gastric secretion. In dogs with pouches of the entire stomach 0.0125 mg. histamine dihydrochloride was given subcutaneously every 10 minutes. When the secretory rate became relatively constant (2 to 3 hours) the drug to be tested was injected intravenously and the histamine injections were continued. Five tenths gm. theophylline was found to potentiate the histamine-stimulated acid secretion (6 tests). The difference was statistically significant. When the theophylline was given alone (without histamine), 3 tests, it did not stimulate gastric secretion. Krasnow in our laboratory has shown that theophylline given alone (intragastrically) in man, *will* stimulate gastric secretion. Xanthiné (0.2 gm.—six tests) and theobromine (0.5 gm.—7 tests) increased the average secretory response to histamine but the differences were not statistically significant. Caffeine has been shown to stimulate gastric acid secretion in cat and man but not in the dog. It also potentiates histamine-induced gastric secretion in cat and man. We have investigated the effect of caffeine on histamine-induced gastric secretion in the dog and found that 0.5 g. (8 tests) potentiated the secretion (statistically significant). These results show that certain xanthine derivatives which, when given alone, fail to stimulate gastric secretion in the dog, are able to augment the response to histamine.

Effect of epinephrine on the water and electrolyte composition of cardiac muscle. WM. V. B. ROBERTSTON and PINCUS PEYSER (introduced by F. J. SICHEL). *Division of Exptl. Medicine and Dept. of Biochemistry, Univ. of Vermont, College of Medicine, Burlington, Vt.* The hearts of anesthetised cats were removed at intervals up to 11 minutes after intravenous injection of 5 to 500 μg. of epinephrine per kilo; and were analysed to determine whether changes in concentration of water and electrolytes in cellular and extracellular phases had occurred. In all cases an increase in extracellular water, as derived from chloride concentration according to the theory of Boyle and Conway (*J. Physiol.* 100: 1, 1941), was found. Direct confirmation of this extracellular increase was obtained by measuring changes in 'sucrose space'. Increases in cell water were also found.

EPINEPHRINE	TIME	$(H_2O)_E$	(H_2O) SUCROSE	$(H_2O)_C$
μg/kg.	*min.*	*m/kg. of dry muscle*		
0		1340		3130
5	4	1590		3020
5	11	1390		3170
50	2	1860		3410
50	4	1800		3300
50	11	1690		3260
500	6	2040		3810
0 (sucrose)		1100	1010	3170
100 (sucrose)	4	1610	1400	3220

An increased sodium and decreased potassium concentration in the cell water was observed only after injection of 500 μg. of epinephrine per kilo.

Factors correlated with resistance to decompression sickness. TRUE W. ROBINSON. *Yellow Springs, Ohio.* In studies made on the resistance to decompression sickness of 18 men in a series of 12 altitude chamber flights, a variety of physiological data was obtained which has now been analyzed for factors which might correlate with this resistance. This data included pre-selection tests for the human centrifuge, accelerational or G-tolerance measurements, basal metabolism determinations, and studies on physiological responses to rest and different kinds of exercise both on the ground and at altitude. In all of the foregoing procedures, information on heart rate, blood pressure, oxygen consumption, and respiratory rate was obtained, as well as personal data regarding weight, height, surface area, anthropometric type, use of alcohol and tobacco, sleeping habits etc. Studies of these various factors indicate that most of these elements show little or no correlation with decompression sickness tolerance (as determined by average time at altitude for 8 or 12 flights). Low correlation is shown by the resting diastolic pressure and the systolic, diastolic and pulse pressures, pulse rate and respiratory rate obtained one minute after a step-up exercise used as pretreatment before a chamber flight. Personal habits and characteristics also do not show a good correlation. There is a fair correlation with the resting pulse rate and a good correlation with resting systolic blood pressure and respiratory rate.

Blood pressure changes produced by local thermal stimulation of the brain. S. RODBARD, L. TAYLOR (by invitation) and D. FERGUSON (by invitation). *Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago, Ill.* The effect of various degrees of thermal stimulation of the brain on the blood pressure response was tested in 248 trials on 50 turtles. After removing the calvarium, a silver wire attached to a water reservoir was inserted into the brain. Various regions were explored for temperature-sensitivity as indicated by blood pressure changes.

With large temperature differences (15°C.) between brain and wire, consistent responses were not seen when the wire was in the olfactory lobe, optic lobe, in the medulla or laterally in the cerebral hemispheres. When the wire was in the midline between the cerebral hemispheres, a consistent increase in blood pressure was seen with warming by means of the wire, and a decrease with cooling. With small temperature differences (5°C.) between the wire and the brain, the blood pressure response was slow and less marked.

Direct determination of gas tensions by blood. ALBERT ROOS and H. BLACK (introduced by H. L. WHITE). *Depts. of Surgery and Physiology, Washington Univ. School of Medicine, St. Louis, Mo.* A study was made of conditions affecting the accuracy of direct *in vitro* determinations of gas tensions in blood. Human blood with heparin and NaF added was equilibrated for 30–60 minutes at 37°C. in a large flask containing known gas mixtures. It was then anaerobically transferred to a small tonometer containing mercury and a small gas bubble, which was mechanically agitated at 37°C. The total bubble tension was the determined with a special capillary manometer, keeping the bubble volume constant, after which the bubble was analyzed in a Scholander apparatus. Calculations showed that, if complete equilibration were accomplished with the bubble volumes and gas mixtures used, the blood oxygen tension should not change by more than 1–4 mm. Hg. However, although the oxygen tensions of blood and bubble approached each other rapidly during the first 10 minutes of equilibration, the exchange slowed down considerably between 10 and 15 minutes in such a way that the bubble tension after 15 minutes still differed from that of the blood by 5–10 mm. Hg., when the initial blood-bubble difference was 10–20 mm. Hg. Satisfactory results were obtained only when the initial bubble tension was within 5 mm. Hg of the blood tension. In bloods with oxygen tensions of 100 mm. Hg and above the tension values fell progressively on repeated determinations, which could not be ascribed to the action of mercury amalgam or stopcock lubricant. This fall was less marked at lower tensions.

Effect of immersion hypothermia on cardiac output. FELIX R. ROSENHAIN (by invitation) and K. E. PENROD. *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Cardiac output has been determined by the Fick method in dogs immersed in cold water. Observations were made before immersion and at rectal temperatures of 35°, 30°, 25° and, where spontaneous breathing continued, 20°C. In 3 experiments early signs of shock developed as evidenced by a precipitous fall in blood pressure and O_2 consumption. These were eliminated from the averages. Data from the other 9 experiments appeared to indicate: *1*) between 35° and 25°C. rectal temperature the average cardiac output, pulse and O_2 consumption fell approximately linearly; *2*) a peak in the O_2 consumption curve at 35°C., associated with shivering, was reflected in the cardiac output curve, but not in the pulse curve; *3*) individual variation in stroke volume was considerable but in most instances there occurred a slight increase with decreasing body temperature.

Histamine as a possible mediator for cutaneous pain. SOL ROY ROSENTHAL. *Univ. of Illinois College of Medicine, Chicago, Ill.* Thin slices of freshly obtained human skin were studied *in vitro* by a method described by Rosenthal and Mennard. Such stimuli as punching, pricking, electrical and burning yielded a profusate which when reinjected into the skin of the donors elicited pain responses, wheal, and flare directly proportional to the degree of stimulus and corresponded closely to histamine injections. The anti-histamine drug N-dimethyl-amino-2-propyl-1-thiodiphenylamine (RP3277) when administered to humans in 40 to 80 mg. single doses resulted after 3 to 4 hours in a diminution of the responses to pain as well as wheal and flare to histamine.

Measurement of sympathetic denervation in man by sweating patterns after sympathectomy. GRACE M. ROTH and WINCHELL MCK. CRAIG (by invitation). *Section on Clinical Physiology and Section on Neurologic Surgery, Mayo Clinic, Rochester, Minn.* Over a 10-year period, 1937 sweating tests were carried out by the cobalt chloride method on 1022 patients 8 to 14 days after surgical interruption of sympathetic fibers in treatment of hypertension or peripheral vascular disease. The sweating test immediately after such operations on the sympathetic nervous system demonstrated irregular patterns of sweating in a large group of patients. This observation indicates incomplete denervation of the region which the operation was expected to denervate rather than regeneration. This incompleteness of denervation probably can be explained by the unpredictable lack of uniformity of the distribution of the sympathetic nerves. Tests five to six months after operation in some instances demonstrated that the region of anhidrosis was less extensive than it had been immediately after operation. After this interval of six months, however, the region of anhidrosis remained practically the same for sixteen to twenty years.

In-vitro histamine release from blood cells of sensitized rabbits—relationship to blood-coagulation mechanisms. L. W. ROTH, FLOYD C. MCINTIRE (by invitation), and R. K. RICHARDS. *Depts. of Pharmacology and Biochemistry, Abbott Research Laboratories, North Chicago, Ill.* The *in-vitro* addition of antigen to whole blood of

sensitized rabbits causes histamine to be released from the cells into the plasma (Katz, *Science* 91: 221, 1940). It is well known that coagulation of blood also causes the release of histamine from the blood cells. In a study of the mechanism of the 'anaphylactic' *in-vitro* histamine release from rabbit blood cells, the possibility of participat on by various components of the blood coagulation system must be considered. Our investigations indicate that thrombin, prothrombin and at least part of the 'thromboplastin complex' probably are not involved in the *in-vitro* 'anaphylactic' histamine release because: *1*) the quantity of heparin required to inhibit only partially the h stamine release by antigen is many times the amount required to prevent blood coagulation under identical conditions; *2*) extreme prolongation of the blood-clotting time of sensitized rabbits by dicumarol treatment does not impair the 'anaphylactic' histamine release; *3*) soybean trypsin inhibitor (Kunitz, *J. Gen. Physiol.* 30: 291, 1947), which is a potent 'anti-thromboplastin' (MacFarlane, *J. Physiol.* 106: 104, 1947), fails to inhibit the histamine release by antigen. However, a similarity between the mechanism of histamine release by antigen and the blood-coagulation mechanism is suggested by the fact that the histamine release by antigen is completely inhibited by .006 molar sodium oxalate or .006 molar sodium citrate and is markedly inhibited by .03 molar magnesium chloride or .02 molar calcium chloride. The citrate inhibition is only partially reversed by recalcification.

An indication that the ovulating hormone release inducing action of progesterone is an indirect one. IRVING ROTHCHILD (introduced by D. C. SMITH). *Bureau Animal Industry, Beltsville, Md.* Progesterone induces ovulation in the intact domestic hen, but is completely ineffective in the absence of the pituitary (Rothchild and Fraps, *Endocrinology* 44: 1949). In order to determine whether it acted directly on the pituitary, 0.012–0.018 cc. of either 10.0 mg/cc. or 1.0 mg/cc. progesterone in propylene glycol was applied to the surface of the pituitary, exposed as for hypophysectomy, in birds carrying a mature follicle, and either left in the drill hole or removed after 1.0 or 3.0 minutes. Out of 10 birds treated with 10.0 mg/cc. progesterone (not removed) 6 ovulated, but only 5 out of 17 treated for 3 minutes, and only 3 out of 8 for one minute ovulated. No birds ovulated following treatment with the 1.0 mg/cc. progesterone for 3 minutes. Practically the same results were obtained when the progesterone (10.0 mg/cc.) was applied either to the partially drilled, or completely undrilled, bone over the pituitary. Two out of 3 birds in which the progesterone was left, and 2 out of 10 treated for 3 minutes ovulated. These findings indicated a relation between passage of progesterone through a systemic pathway and effectiveness in inducing hormone release from the pituitary. Additional weight to this possibility was given by the fact that 7 out of 10 birds ovulated following application of 3 drops of the 10.0 mg/cc. progesterone to the thigh muscle for only 3 minutes.

Some factors influencing the exchange of ions across nerve membranes. MORTIMER A. ROTHENBERG (introduced by DAVID NACHMANSOHN). *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.* The ion exchanges across the nerve membrane have been measured by direct determination of K^{42} and Na^{24} penetration into the axoplasm of the giant axon of Squid. Previous results indicate that at rest the ionic composition in these nerves is in dynamic and not static equilibrium with that of their outer environments. Employing the same technique, several physical and chemical factors influencing this equilibrium have now been studied. Changes in temperature showed a small Q_{10} supporting the assumption that at rest there are no important chemical reactions involved other than a Donnan equilibrium. Electrical activity markedly increased the rate of Na^{24} penetration. Anticholinesterases, in general, gave an overall result similar to that obtained with nerve activity. Exposure to diisopropyl fluorophosphate increased the rate of Na^{24} penetration by about 50% and decreased the K^{42} penetration by about 35%. The increase in Na^{24} penetration produced by eserine was slightly smaller. Unlike the anticholinesterases, cocaine had a very small effect on the membrane permeability. Exposure of nerves to high doses of X-rays produced a marked increase in Na^{24} penetration and a marked decrease in K^{42} penetration in normally conducting nerves. This observation indicates an early phase of possible damage to the intact nerve cell by irradiation in spite of apparently normal function.

Action of thyroxine on protein metabolism. J. RUPP (by invitation), K. E. PASCHKIS and A. CANTAROW. *Jefferson Medical College, Philadelphia, Penna.* Thyroid hormone is usually looked upon as stimulating protein catabolism. However, certain well-known facts, such as the dwarfed stature of cretins and the resumption of growth following thyroid therapy, indicate that thyroid hormone may exert a protein anabolic effect. The following experiments were performed in order to determine under what circumstances thyroid hormone would be a 'protein anabolizer.' All experiments were performed on force-fed rats receiving a constant food intake. Thyroidectomy resulted in N-loss; this was observed as a late effect, thereby precluding unspecific negative N-balance due to post-operative alarm reaction. Daily injection of 5 to 10 μg. thyroxine (physiological range) was followed by N-retention (ana-

bolic effect) in the thyroidectomized rat, but had no effect in the intact rat. Large doses (50–100 μg.) caused N-loss (catabolic effect) both in intact and in thyroidectomized rats. In hypophysectomized rats neither the anabolic effect of small, physiological amounts, nor the catabolic effect of large doses of thyroxine could be obtained. Pituitary function appears to be necessary for the effect of thyroid hormone on protein metabolism in fed rats. Experiments are under way to investigate further the interaction of thyroid and pituitary in protein metabolism.

Cinéfluorography of the normal human heart; teaching application (motion picture). R. F. RUSHMER (introduced by T. C. RUCH). *Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, Wash.* A training film demonstrating the anatomical relations of the normal cardiac silhouette in the postero-anterior, right anterior oblique, and left anterior oblique positions is presented. Heart models, teleroentgenograms and cinéfluorograms are arranged in sequence to facilitate visualizing the fluoroscopic appearance of the heart.

Kinetics of cholinesterase of erythrocytes in pernicious and other anemias. JEAN CAPTAIN SABINE (introduced by CHARLES I. WRIGHT). *Experimental Biology and Medicine Inst., National Insts. of Health, Bethesda, Md.* Cholinesterase activity was determined manometrically over a wide range of concentrations of acetyl choline in 4 cases of pernicious anemia and 5 cases of anemia from hemorrhage. The data were subjected to the analysis previously made of normal human arythrocytes (*J. Pharm. & Exper. Therap.* 93: 230, 1948). The theoretical limiting velocity, V, expressed per unit volume of red blood cells (or per cell), was increased by a factor of 1.5 to 2.5 in anemia from hemorrhage and in pernicious anemia in relapse and during treatment. The values of the apparent dissociation constants K_s and K_2 of the active and inactive enzyme-substrate complexes were normal in anemia from hemorrhage. In pernicious anemia in relapse the experimental value of K_s was increased about 3-fold and that of K_2 decreased by the same amount. During three weeks or more of treatment the experimental values of these constants gradually shifted to normal. This shift is probably not attributable to a direct action of the therapeutic agent on the enzyme-substrate system. The high value of V was maintained until the red count and hematocrit approached the normal levels and then fell off. Discussion will include the interpretation of the changes found in pernicious anemia, interpretation of previous findings in pernicious anemia in the light of the present investigation, and a correlation which has been found between clinical condition of severely anemic patients in general and increased enzymic concentration in the red cells.

Analytic study of effects of various patterns of X irradiation on survival. GEORGE A. SACHER, JOHN SACKIS (by invitation) and AUSTIN M. BRUES. *Biology Division, Argonne National Laby., Chicago, Ill.* Sixteen hundred female CF-1 mice were treated with 250 kv. total-body X radiation according to the following dosage patterns: 400 r in a single dosage, or in 10 or 40 daily treatments; 800 r in 10 or 40 treatments; 1200 r in 40 treatments, 5 r daily throughout life; 800 r in 40 treatments superimposed upon 5 r daily throughout life. There were 1400 control mice. Gross mortality rates have been computed as a function of time, in addition to rates associated with certain prominent types of pathologic change. As a general rule, all patterns of treatment increase the incidence of all types of pathology at all ages. The effect of radiation on rates of mortality is best expressed by means of the Gompertzian diagram (logarithm of rate of mortality against time). In this form, all categories of pathology in control animals show a linear increase with age, as is well known in the case of many human diseases. A dosage administered to young adult mice within a short interval (weeks) displaces these lines upward by a constant amount. The incidence of lymphoid tumors is phasic and constitutes an exception to this rule. The effect of daily small exposures throughout life combined with a heavy exposure early in life is discussed in terms of gross mortality exlcuding lymphoid tumors. To a first approximation, the results are additive on the Gompertzian diagram. The results indicate lines along which a calculus of radiation mortality may be developed for arbitrary time patterns of exposure.

Turnover rates of acid-soluble phosphorus compounds of liver. JACOB SACKS and SHEPHERD STIGMAN (by invitation). *Biology Dept., Brookhaven National Laby., Upton, L. I., N. Y.* Methods have been worked out for the separation of various acid-soluble phosphorus compounds of liver and applied to tracer experiments on rats in the post-absorptive state. The time-course of the P^{32} levels of plasma inorganic-P and the organic-P compounds of liver has been studied. The highest P^{32} level in the plasma P was found ½ hour after subcutaneous injection of the tracer phosphate. The level fell rapidly for the next 1.5 hours, and then progressively more slowly. The maximum P^{32} levels in the P compounds of liver were generally found 1.5 hours after injection of the tracer. The labile P of ATP and ADP showed a higher turnover rate than any other organic P compound studied. The time-course of the P^{32} content of the intracellular inorganic P of liver was found to run parallel with that of the labile P of ATP and ADP,

and to show no such relation to that of plasma P^{32}. This indicates that the former compounds serve as the principal means of transferring inorganic phosphate from the extracellular phase to the interior of the liver cell. Data on the relative turnover rates of ATP-ADP and of glucose-1- and glucose-6- phosphates are presented.

Interplay of light and heat in bleaching rhodopsin. ROBERT C. C. ST. GEORGE (introduced by GEORGE WALD). *Biological Laby. of Harvard Univ., Cambridge, Mass.* The pigment of rod vision, rhodopsin, is destroyed by heat with an apparent activation energy for this process of about 45,000 cal/mol at *p*H 5–9 (Lythgoe and Quilliam). At lower temperatures it bleaches in white light by a process independent of the temperature (Hecht). In the present experiments an attempt was made to study the transition between these situations by irradiating rhodopsin with red light, quanta of which are in themselves too small to supply the entire energy of activation. It was found that at *p*H 7 the bleaching of rhodopsin by light has become appreciably temperature-dependent at 650 mμ. As the wave length is increased further the temperature coefficient of bleaching continues to rise. The apparent thermal energies of activation found at these long wave lengths, added to the energy content of the light itself, appears to approximate 45,000 cal/mol. That is, when less than 45,000 cal. are supplied by the absorbed light quanta, the deficit in activation energy is made up from the internal energy of the molecules themselves. About 45,000 cal. appear to be required for bleaching rhodopsin, whether supplied as light or heat or as a combination of both. Measurements could not be made at wave lengths longer than 715 mμ, due to the very low absorption of rhodopsin in this region. These observations suggest however that at 1050 mμ, where rod vision still persists (Griffin, Hubbard and Wald) and where the energy content of the radiation is only about 27,000 cal/mol of quanta, about 18,000 cal. probably come from the internal energy of the rhodopsin molecule.

Phosphate turnover in the mammalian nervous system. A. J. SAMUELS (by invitation), L. L. BOYARSKY (by invitation), B. LIBET, R. W. GERARD, M. BRUST (by invitation). *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* Radioactive phosphorus, 300 microcuries as NaH_2PO_4, was injected intraperitoneally into guinea pigs in a single dose or spread over 2 weeks in daily doses. Animals were killed at intervals from 6 hours to a week over a 5-week span and the brain, spinal cord and sciatic nerves analyzed for acid-soluble, phospholipid, phosphoprotein, and nucleoprotein phosphorus following Schneider, and activity measured. The time course of specific activities (counts/min/μg. phosphorus), especially the ratio (R) of activity of a tissue sample to that of inorganic P in plasma or tissue at a given time, indicate rates of penetration or turnover. The uptake of phosphate by nervous tissue from plasma is slow; R (for tissue acid-soluble P against plasma) reaching unity (an arbitrary value) in about 22 days for brain, 30 days for nerve and 36 days for spinal cord. Turnover rates were also slow. Bracketed numbers below give average mg. % P of tissue fraction indicated. R (against tissue acid-soluble P) for phospholipid reaches unity in 32 days in brain (166) and spinal cord (341), 37 days in nerve (254); for phosphoprotein, 30 days in spinal cord (26), 36 days in brain (21), 40 plus days in nerve (26); for nucleoprotein, 16 days in cord (16), 40 plus days in brain (24) and nerve (17). R for phosphoprotein rises faster in proximal segments of nerve than in distal ones. Our evidence favors the view that protein moves continuously from cord to periphery at a rate of about 3 mm/day. A mathematical method has been developed for describing certain simple cases of the uptake and leakage of radioactive materials by tissues.

Hormones and hormone-like substances in the treatment of (experimental) peptic ulcer. DAVID J. SANDWEISS and HARRY C. SALTZSTEIN (by invitation). *Harper Hospital and Dept. of Surgery, Wayne Univ. College of Medicine, Detroit, Mich.* This report deals with the results obtained from 37 control (untreated) Mann-Williamson dogs and 12 other series of similarly operated animals treated with various hormones and hormone-like substances, totaling in all 282 animals. All preparations were administered parenterally. In addition, enterogastrone and the anterior pituitary-like hormone (Ant-S) were also administered orally in two other series of Mann-Williamson dogs. The average post-operative survival time of the control series was 63 days. All control animals died of ulcer. The longest survival time was 135 days. Eight % of the ulcers showed microscopic evidence of healing (epithelization). Results in the treated series of animals are as follows: *1*) The extracts obtained from human urine (pregnant women, normal women and normal men) were of definite benefit. Extracts obtained from the urine of ulcer patients had some, but very much less activity. Pregnancy urine extract administered orally did not prolong the life of the animals but it did stimulate epithelization in a significant number of the ulcers. *2*) The gonadotropic hormone from pregnant mare's serum (Gonadogen) was definitely beneficial. *3*) Progesterone (Proluton) showed some beneficial effect, in that it definitely stimulated epithelization. *4*) Estrogen (Theelin) was of no value. *5*) The posterior pituitary extract (Pituitrin) produced no effect; a combination of Pituitrin and Ant-S warrant further study. *6*) Enterogastrone administered orally produced some benefit but when it was administered parenterally,

the enterogastrone concentrate we employed had no beneficial effect.

Concerning relation of the pituitary gland to enterogastrone and urogastrone. D. J. SANDWEISS, J. KAULBERSZ, T. L. PATTERSON and H. C. SALTZSTEIN (by invitation). *Depts. of Physiology and Surgery, Wayne Univ. College of Med. and Harper Hosp., Detroit, Mich.* Enterogastrone and urogastrone were prepared from the intestines and urine of normal dogs and hypophysectomized dogs by the usual methods. The procedures were identical for both series of experiments. The effect of these extracts on gastric secretion was studied by the double-histamine tests on fistula and Heidenhain pouch dogs. The extracts were always administered during the first period of the test. Approximately an equal degree of inhibition of gastric secretion was observed with both normal enterogastrone (12 exper., 4 dogs, dose 50 mg.) and hypophysectomized enterogastrone (8 exper., 3 dogs, av. dose 23 mg.). Normal urogastrone (32 exper., 7 dogs, dose 1 to 2 mg.) and hypophysectomized urogastrone (16 exper., 5 dogs, dose 3–10 mg.) also produced a comparable degree of inhibition in response to histamine as an immediate effect. However, while no after-effect could be detected in the experiments with normal urogastrone a more striking inhibitory effect in comparison with the immediate effect was noted 5 to 9 hr. after administration of the hypophysectomized urogastrone. These effects were found to be statistically significant. It appears, therefore, that hypophysectomy does not significantly alter enterogastrone formation. However, the characteristics of urinary extracts are changed by hypophysectomy in a very striking manner as noted above and as described more fully in another presentation (see Patterson *et al.*).

The effect of spinal anesthesia on circulating blood volume. S. J. SARNOFF, E. S. BUCKLEY, JR., J. L. WHITTENBERGER and J. G. GIBSON, II (introduced by W. H. FORBES). *Dept. of Physiology, Harvard School of Public Health, and Dept. of Medicine, Harvard Medical School, Boston, Mass.* In preliminary clinical studies it was demonstrated that spinal anesthesia benefited the patient in pulmonary edema. Subsequent clinical studies confirmed these early observations. It was thought that one of the associated phenomena that accompanies spinal anesthesia might be a reduction in the volume of blood in the pulmonary vascular bed. As a result of plethysmographic and oncometric studies in which the volume of the extremities and the viscera is found to be increased during spinal anesthesia, it is assumed that the volume of blood in the systemic circuit is increased during this type of sympathetic block. It was decided therefore that if, under spinal anesthesia, the total blood volume remained unchanged, the volume of blood in the pulmonary bed should be diminished. Total circulating plasma volumes were determined by the T-1824 method and red cell volumes were determined by the radioactive red cell technique just before spinal anesthesia and 30 and 60 minutes after it was started. Spinal anestheisa was administered through an indwelling ureteral catheter previously introduced through the atlanto-occipital membrane. Spinal anesthesia adequate to obliterate the tendon reflexes and cause lower intercostal paralysis was maintained by intermittent injections of 2% procaine hydrochloride. The total circulating plasma volume was found to be unchanged in 6 dogs under the influence of spinal anesthesia.

Effects on the circulation of electrophenric respiration and positive pressure breathing during high spinal anesthesia. STANLEY J. SARNOFF, JAMES V. MALONEY, JR. and JAMES L. WHITTENBERGER (introduced by WILLIAM H. FORBES). *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.* It was demonstrated that intermittent positive pressure depresses the circulation of the dog when spinal anesthesia is carried high enough to cause respiratory paralysis. Under these conditions, the sympathetic vasoconstrictor outflow is totally blocked and increased intrathoracic pressure, even though it be intermittent, retards the return of blood to the heart. Proportionality was established between the pressure used during insufflation and the depression of the blood pressure. Electrophenric respiration was accomplished by applying a cyclically undulating potential to one or both phrenic nerves so that the diaphragm was made to contract and relax in a smooth manner. When electrophrinic respiration was applied, the effect was a stimulation of the circulation in the same animals whose blood pressure was depressed by positive pressure breathing. Changing from positive pressure breathing to electrophrenic respiration in the dog under total spinal anesthesia widens the pulse pressure and elevates the mean arterial blood pressure between 25 and 40 mm. Hg. Changing from electrophrenic respiration to positive pressure breathing depresses the blood pressure by the same amount. Under the conditions of the experiment, in which there is a total sympathetic block, elevations or depressions of blood pressure are taken to reflect similar qualitative changes in cardiac output.

Effect of pitressin and desoxycorticosterone in low dosage on excretion of water and sodium. OTTO W. SARTORIUS and KATHLEEN ROBERTS (Introduced by R. F. PITTS). *Dept. of Physiology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* In a series of experiments on 3 normal female dogs in which a standard 2-hr. urine flow curve was obtained following the oral administration of 40 cc. of water/kg., the intravenous injection of 0.8 m.u. of pitressin/kg. produced an antidiuresis of

30–45 minutes duration. The antidiuresis so obtained was substantially modified by intravenous injection of small doses of homogenized desoxycorticosterone in sesame oil and water, administered 15 minutes prior to the pitressin and at the same time as the water load. All experiments were performed under similar fasting conditions with all control urine flows ranging between 0.2 and 0.5 cc/min. Urine collections were obtained by catheter at 15-minute intervals according to standard technique. Sodium excretion, determined in all experiments, and within limits of 5 and 25 μEq/min. in the control periods, decreased to about one-third during water diuresis returning to the original level 2–3 hrs. later. Pitressin in a dosage of 0.8 m.U/kg. produced a 10-fold increase in sodium excretion with the peak excretion occurring 45 minutes after its administration. By 90 minutes, sodium excretion had fallen below the control water diuresis level. The effect of pitressin on sodium excretion was modified or abolished by 10 to 100 γ/kg. of desoxycorticosterone. It is probable that pitressin and desoxycorticosterone are antagonistic in physiological dosage both as to water excretion and sodium excretion.

DFP and reflex activity in salamander larvae. CHARLES H. SAWYER (introduced by J. E. MARKEE). *Dept. of Anatomy, Duke Univ., Durham, N. C.* Di-isopropyl fluorophosphate (DFP) which irreversibly inhibits cholinesterase (ChE), also severely impedes reflex activity in salamander larvae (Boell, 1946). The present experiments were devised to determine what degree of correlation existed between the amount of enzyme inhibition and the extent of interference with reflex motility. Fifty larvae of Amblystoma punctatum (Harrison's stage 46) were stimulated individually with a hair 60 times at one-second intervals and the number of each animal's reflex responses was recorded. Twenty-five larvae were then immersed in solutions of DFP (5.5×10^{-5} M) for intervals varying from 5 minutes to several hours. At the end of its DFP period each experimental larva was stimulated and scored as above, washed, and assayed for ChE activity. Controls were clutch-mates of the DFP animals and reared under identical conditions but for the anti-cholinesterase; each control had a reflex-response score closely similar to that of its experimental counterpart prior to the latter's immersion in DFP. A high degree of positive correlation was found to exist between the residual reflex capacity (expressed as percentage of the pre-inhibition response score; range, 0–100%) and the residual ChE activity (expressed as percentage of the control's enzyme content; range, 0–99%): The coefficient of correlation, $r = 0.93$. It is concluded that the effect of DFP on reflex motility is attributable entirely to inhibition of ChE and that a quantitative relationship exists between reflex capacity, during a given developmental stage, and the amount of cholinesterase available to the neuromuscular apparatus.

Quantitative estimation of capillary passage of fluorescein in rabbit skin. ALFRED A. SCHILLER. *Dept. of Physiology, Univ. of Ill., College of Medicine, Chicago, Ill.* The fluorescence of minute concentrations of sodium fluorescein in the skin can be measured photometrically in long wave UV. The time and intensity relations of the appearance and disappearance of the dye from wheals made with vasoactive drugs have been found to be sufficiently graded to establish quantitative response patterns characteristic of the drug action at a given concentration. Serial dilutions of histamine phosphate, epinephrine HCl, and histamine + epinephrine dissolved in 0.9% NaCl solution were used to make the wheals in the abdominal skin of the rabbit. Controls were performed with the solvent alone. Fluorescein was either injected intravenously or incorporated in the wheal solution (0.1 ml. intradermally). The results were as follows: the intensity of fluorescence increased and the disappearance rate of the dye decreased with increasing concentration of histamine over a range of 10^{-3} to 10^{-7}. Epinephrine concentrations from 10^{-3} to 10^{-6} prevented fluorescence in the wheals. The diameters of the non-fluorescent wheals were increased with drug concentration. By using various ratios of simultaneously injected mixtures of histamine and epinephrine it was found that histamine 10^{-3} + epinephrine 10^{-5} produced the same response as the saline control. This method may be of use for the study of capillary permeability when correlated with data supplied by other techniques.

Reduced evaporative water loss from the lungs of certain desert mammals. BODIL SCHMIDT-NIELSEN (introduced by LAURENCE IRVING). *Dept. of Physiology, Stanford Univ., and Arctic Research Laby., Stanford, Calif.* It was shown that desert rodents of the family Heteromyidae are able to economize with water by excreting a highly concentrated urine. The question arises if they have other mechanisms for saving water. The evaporation from the animal was determined simultaneously with the oxygen uptake. When the water evaporated is calculated on the basis of the simultaneous oxygen consumption it is found that the water evaporation is approximately one half of what it is in other rodents of the same size (white rat and white mouse). The water evaporation from the Heteromyids breathing completely dry air is on the average 0.45 mg. water/ml. oxygen taken up. The amount of water formed by metabolism of food in the body is approximately 0.65 mg. water/ml. oxygen consumed. Therefore water is gained in the metabolism, even when dry air is

inspired. (See also abstract by Knut Schmidt-Nielsen.)

Efficiency of the excretory system in desert mammals. KNUT SCHMIDT-NIELSEN (introduced by LAURENCE IRVING). *Dept. of Physiology, Stanford Univ., and Arctic Research Laby., Stanford, Calif.* The critical factor in the water metabolism of mammals living in deserts is that the expense of water required for urine, saturation of the expiratory air, and heat regulation cannot easily be covered by the limited supply of water available. Reduction of any of these three main sources of water loss will be of advantage to the organism. Desert rodents of the family Heteromyidae live without access to drinking water. We found that their renal excretory system is well adapted to conserve water by a very concentrated urine (1 normal salt and 4 molar urea). To prove that high urine concentrations can be maintained and are not only occasional, we gave sea water (ca. 0.58N) for drinking. In view of the capacity for concentrating urine it might be expected that the animals would tolerate sea water, which other mammals cannot because of its large salt content. It was found that the Heteromyids do tolerate drinking sea water, and are even able to excrete simultaneously excessive amounts of non-electrolytes (urea). (See also abstract by Bodil Schmidt-Nielsen.)

'Impedance' variation in the nerve membrane during passage of the natural action potential. OTTO H. SCHMITT. *Dept. of Zoology and Physics, Univ. of Minnesota, Minneapolis, Minn.* Extension of a previously described method of analysis permits direct and semi-quantitative calculation of the characteristic impedance-source function for the active nerve membrane during the passage of a normal impulse without superposition of any testing current. This total function can be separated into two parts, a passive component which represents the quiescent physical-chemical properties of the membrane, and an active or 'live' component which includes the energy-yielding mechanism of the impulse. Using experimental results quoted in the literature supplemented by data from new experiments, it has been possible to evaluate, with relatively low accuracy, the passive part of the function, and from this to work back to an approximate numerical evaluation of the active part of the function. By measuring all of the critical quantities involved in the analysis for a single type of nerve, or preferably for an individual specimen, it should be possible to improve greatly the quantitative accuracy of the results. Accurate values for the impedance-source function should prove useful in testing thermodynamically adequate theories for the conversion of biochemical energy to bioelectrical energy.

Action potentials of the cerebellar cortex elicited by stimulation of olivo-cerebellar fibers. GORDON M. SCHOEPFLE. *Dept. of Physiology, School of Medicine, Washington Univ., St. Louis, Mo.* Cerebellar action potentials are recorded by means of a unipolar lead (microelectrode) at various levels within the cortex of the dog in response to single shock stimulation of olivo-cerebellar fibers. The stimulating needle electrodes are thrust into the brain stem from above in the region of the inferior olivary nucleus. Voltage changes associated with post synaptic responses when recorded between folia or in the molecular layer of each folium tend to be manifest as several successive positive deflections. At regions containing the Purkinje cell layer, granular layer, or central white lamina the successive post synaptic responses exhibit pronounced negative components. In fact the first post synaptic action potential may show no initial positivity. Such a series of results is reproducible from folium to folium as the recording needle electrode is progressively thrust deeper into the cortex at an acute angle with the plane of the brain surface. Measurements of electrode position taken in connection with histological examination of the cortex appear to be precise to within approximately three tenths of a millimeter. The phase reversals observed are consistent with considerations of volume conductor theory which include the concept of neurones as polarized cylindrical structures which, however, do not undergo depolarization in terminal regions at any time during activity. No phase of any particular response persists for more than a few milliseconds and no underlying slow potential waves are apparent.

Rapid spontaneous variations in blood pressure. HENRY A. SCHROEDER. *Dept. of Internal Medicine, Washington Univ. School of Medicine and Barnes Hospital, St. Louis, Mo.* When blood pressure in the brachial artery of man is measured by Hamilton's optical manometer, spontaneous variations occur from beat to beat which may be of relatively considerable magnitude. Fifty records of blood pressure of normotensive and hypertensive subjects and those with coarctation of the aorta were examined for these variations. In half the difference in systolic pressure from one beat to an adjacent one was 10 mm. Hg or more. The average systolic difference was 10.3 mm. Hg; the average diastolic 7.0. Various patterns were seen; in some cases respiratory variations were marked, in others two or three beats with high pressure were followed by two or three with lower. In several cases similar variations were found in records made of pressure in the femoral artery. Hypertensive patients on the whole showed greater variations in beat to beat pressure than did normotensive ones, and there was usually little respiratory variation. These changes could also be detected by

the auscultatory method when the pressure cuff was inflated exactly to systolic pressure.

Absence of antifibrillatory effect in quaternary nitrogen compounds. JOHN E. SCHULTS (by invitation) and JOSEPH R. DIPALMA. *Depts. of Physiology and Medicine, Long Island College of Medicine, Brooklyn, N. Y.* The activity of a compound as an antifibrillatory drug was determined by means of a thyratron stimulator set at 600 impulses/min. and connected through a rheostat to the right auricle of an anesthetized open chest cat. The minimal amount of current, in milliamperes, necessary to induce fibrillation was determined before and after the administration of a drug. The degree of difference was the index to the activity of the compound. The drug to be tested was administered in a dose of 5 mg/kg. i.v. and the fibrillation threshold determined immediately and at10-and 20-minute intervals. Dawes (*British J. Pharm. & Chemotherapy* 1: 90, 1946) noted in testing various quinoline derivatives for antifibrillatory activity that, when their tertiary nitrogen was changed to a quaternary one, these compounds lost their potency. A recently synthesized compound, N-methyl-N-(3,4, dimethoxybenzyl- -(4 methoxyphenyl)-ethylamine hydrochloride, is a potent antifibrillatory agent. When the tertiary nitrogen of this compound is converted to a quaternary one by the addition of a methyl and halogen groups the activity is lost and in some respects the compound now resembles muscarinic drugs. Other quaternary nitrogen compounds such as tetraethyl ammonium chloride, dibenamine and mecholyl, some of which are related to antifibrillatory drugs, were tested by this method and found to have no antifibrillatory activity and some even lowered the fibrillation threshold.

A method for determination of the volume of distribution of metabolized substances. IRVING L. SCHWARTZ, MURRAY RABINOWITZ and DAVID SCHACHTER (introduced by HOMER W. SMITH). *Dept. of Physiology, New York Univ. College of Medicine, New York City.* Following an intravenous injection of mannitol, Newman, Bordley and Winternitz (*Bull. Johns Hopkins Hosp.* 75: 253, 1944) utilized the relationship between *1*) renal clearance, *2*) volume of distribution, and *3*) decrement in plasma concentration with time to determine *1*) and *2*). It is now known (*J. Lab. & Clin. Med.* 32: 192, 1947; *Proc. Soc. Exptl. Biol. & Med.* 66: 62, 1947) that mannitol is metabolized. In order to measure the total clearance (renal and metabolic), the rate of infusion (IV) required to maintain a constant plasma level was determined; thereafter the infusion was discontinued and the decrement in plasma concentration with time was observed. Under conditions of constant total clearance, O, the volume of distribution, V_m, was calculated as: $V_m = \frac{O\,(t' - t)}{\log_e \frac{P}{P'}}$, where P and P′ are the plasma concentrations at times t and t′ respectively. It is assumed that P and P′ represent the concentration throughout the entire volume of distribution. In 3 experiments on 2 dogs, the volume of distribution of mannitol determined simultaneously with that of inulin (*Proc. Soc. Exptl. Biol. & Med.* 68: 507, 1948) was as follows:

Mannitol Space		*Inulin Space*	
cc.	*cc/kg.*	*cc.*	*cc/kg.*
4,750	206	4,850	210
4,775	201	4,900	208
3,230	199	3,310	203

Transcapillary exchange of inulin in man. IRVING L. SCHWARTZ, DAVID SCHACHTER and NORBERT FREINKEL (introduced by HOMER W. SMITH). *Dept. of Physiology, New York Univ. College of Medicine, New York City.* The movement of inulin between plasma and interstitial fluid was studied in each of 3 normal men utilizing T_{1824} space as a measure of plasma volume, and inulin space (*Proc. Soc. Exptl. Biol. & Med.* 68: 507, 1948) as a measure of extracellular fluid. Following a single intravenous injection of inulin serial samples of venous blood and urine were collected for 24 hours. The amount of inulin (Z) in the interstitial space at any time (t) following administration may be calculated from the injection dose (D), total cumulative urinary excretion at t, (UV), plasma water inulin concentration ($P_{w_{In}}$) and plasma water volume (P_{w_v}) by the expression $Z = D - UV - P_{w_{In}}P_{w_v}$. The volume of interstitial fluid (I_v) is $ECF - P_{w_v}$; hence Z/I_v represents the virtual concentration of inulin in interstitial fluid if it were mixed evenly throughout that compartment. The simultaneous plasma and interstitial fluid concentrations plotted against time demonstrate: *a*) early rapid movement of inulin from plasma to interstitial fluid; *b*) maximal concentration of inulin in interstitial fluid in 15 to 20 minutes at a value below the plasma concentration; *c*) identity of plasma water concentration ($P_{w_{In}}$) and mean interstitial fluid concentration (Z/I_v) 40 to 50 minutes after injection; *d*) Z/I_v remains higher than $P_{w_{In}}$ thereafter because of the gradient between interstitial fluid and plasma. Two important implications of these studies are: *1*) a single injection of inulin should not be used to determine the volume of distribution since true equilibrium never occurs and mean virtual equilibration of interstitial fluid with plasma water occurs at only one instant in time; *2*) reversal of flow of inulin between plasma and the most avail-

able phases of the interstitial fluid occurs before the point of mean equilibration.

Potentiation of the prolactin effect. ERWIN SCHWENK (by invitation), SIBYLLE TOLKSDORF (by invitation), ROY MCCULLAGH (by invitation) and RICHARD TISLOW. *Biological Research Laby. and the Chemical Research Div. of Schering Corporation, Bloomfield, N. J.* An increase in the effect of anterior pituitary lactogenic hormone preparations (Prolactin) has been observed when Sørensen's phosphate buffer (*p*H 7.4) was added to the aqueous solution of the hormone. The assay used was the crop sac weight response in white Carneau pigeons according to the method of Riddle and Bates. The effect was observed both by subcutaneous and intramuscular routes of administration. When averaging repeated assays of the International Standard and Schering Substandard of the lactogenic hormone, the increase in response to the two standards in the presence of buffer was approximately 2-fold. The effect was noted with Prolactin fractions of various purity and potency ranging from starting material of crude pituitary extracts with a potency of 0.4 IU/mg. to highly purified fractions containing 35 IU/mg. It was observed with phosphate buffer of 0.1, 0.2, 0.4 and 0.6 molarity. The effect was only noticed when the hormone was injected in the buffered solution, and not upon separate injection of buffer and hormone solutions. The effect was not observed when phosphate-buffered Prolactin was dialysed free of buffer, precipitated and redissolved in water. The increased response appeared again upon addition of buffer to the solution. Other buffers, such as acetate and arginine, produced a similar effect.

Response of individual pacinian corpuscles to external stimulation. DONALD SCOTT, JR. (introduced by FRANK BRINK, JR.). *Johnson Foundation, Univ. of Pennsylvania, Philadelphia, Penna.* Single Pacinian corpuscles can be dissected from the mesentary of the cat with the single sensory nerve fibre and circulation uninterrupted. The electrical activity can then be studied by lifting the corpuscle on to a fine cotton wick electrode connected to recording equipment. The corpuscle will respond to steady pressure by a glass rod with a series of impulses; the average frequency of which can be altered by increasing or decreasing the pressure. The threshold value for this form of stimulation lies below 5 mg. The corpuscle is much more sensitive to vibratory stimulation as applied by a fine vibrating glass rod or a pure tone. Treatment of the corpuscle with citrate will increase the excitability to the point of spontaneous activity. Analysis of the length of interval between adjacent impulses after the scheme of Brink, Bronk and Larrabee (*Ann. New York Acad. Sci.* 47: 457, 1946) shows that the corpuscle responds in the manner found by these authors where a fundamental unit interval was shown to separate many impulses while others were separated by simple multiples thereof. The length of the fundamental interval of the corpuscle is 4–5 msecs. Careful examination of photographic records where two or more fundamental intervals separate adjacent impulses shows small oscillations of the base line between impulses which have the same period as the fundamental interval of the conducted impulses. This suggests the existence of a continuous oscillation of the excitability of the corpuscle.

Anaphylactic release of histamine and heparin from the isolated dog liver. A. E. SCROGGIE (by invitation) and L. B. JAQUES. *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada.* The anaphylactic reaction of the livers of dogs sensitized to horse serum was studied by perfusing the organ with the antigen both in situ and isolated. The assay of perfusates for histamine and heparin, before and after perfusion with antigen, demonstrated: *1*) When the liver was perfused in situ the release of large amounts of these substances occurred in 2 of 6 animals. *2*) When the livers were isolated, perfusion with antigen in 'silicone' blood caused a smaller but significant release of histamine and heparin in 6 of 9 animals. *3*) When isolated livers were perfused with antigen in Tyrode's solution no release of histamine or heparin occurred. Subsequent perfusion with blood and the addition of antigen to this blood also caused no release of the anaphylotoxins. *4*) When antigen was perfused in Tyrode's solution through the isolated livers of 2 dogs which had received two sensitizing doses of antigen, as compared with the single sensitizing dose given to the dogs of *1*), *2*) and *3*), a release of histamine and heparin was obtained, of the order of that in *2*). From these results, it is concluded: *1*) it is possible to demonstrate the Schultz-Dale phenomenon with canine tissue, by using the isolated liver and measuring the release of histamine and heparin; *2*) this reaction is assisted by using 'silicone' blood in place of Tyrode's in the perfusion. This is probably due to blood maintaining normal function of liver cells, rather than entering into the anaphylactic mechanism resulting in the liberation of the anaphylotoxins.

Metabolism in colpidium campylum. GERALD R. SEAMAN (introduced by CHARLES G. WILBER). *Biological Laboratory, Fordham Univ., New York City. Colpidium campylum* rapidly utilizes acids of the tricarboxylic acid cycle. The addition of pyruvate results in an increased oxygen consumption of 120%; α-ketoglutarate of 102%; fumarate of 90%; malate of 97%; oxaloacetate an increase of 85%. Added citrate causes no increase in oxygen consumption. Malonate inhibits the oxidation of pyruvate in *Colpidium*. Fumarate, succinate, and

α-ketoglutarate release the inhibition. Citrate, however, does not release the malonate inhibition. Malonate inhibition in the presence of citrate is released by fumarate to approximately the same extent as when citrate is absent. Fumarate and α-ketoglutarate are recovered when colpidia are incubated with pyruvate. The addition of fumarate to pyruvate increases the recovery of α-ketoglutarate by 140%. Pyruvate, fumarate, and lactate are recovered when oxaloacetate is the sole substrate. As a result of fumarate release of malonate inhibition, there is an added utilization of pyruvate and an added recovery of succinate. The amounts of cytochrome C, DPN, and glutathione in *Colpidium* are (in γ/mg. dry weight of cells) respectively: 0.17, 0.82, 1.08. The ATP-ase activity is 4.7γ inorganic P liberated/15 min/mg. dry weight of cells. Transamination reactions in Colpidium will be reported.

Effect of 933F on the action of dibenamine. JOHN C. SEED AND ELIZABETH A. MCKAY (introduced by J. H. WILLS). *Pharmacology Section, Medical Division, Army Chemical Center, Md.* Inasmuch as 933F (piperidinomethyl-3-benzodioxane) and dibenamine (dibenzyl-β-chlorethylamine) both block the pressor action of epinephrine, it was felt that the two drugs might act by way of the same mechanism. If this were true, one should be able to prevent the action of dibenamine with 933F. Five hours after the administration of 5 mg/kg. of 933F to the nembutalized dog, 5 μgm/kg. of epinephrine causes a marked pressor response. Five hours after the administration of 10 mg/kg. of dibenamine, 5 μgm/kg. of epinephrine causes a marked depressor response. Five hours after the administration of 5 mg/kg. of 933F followed in 3 min. by 10 mg/kg. of dibenamine, 5 gm/kg. of epinephrine causes a marked pressor response which is of the same magnitude as if dibenamine had not been given at all.

Electrolyte metabolism following massive glucose, mannitol, urea infusions in normal subjects during hydration and dehydration. DONALD W. SELDIN AND ROBERT TARAIL (introduced by DONALD H. BARRON). *Dept. of Internal Medicine, Yale Univ. School of Medicine, New Haven, Conn.* Following glucose or mannitol infusion, serum sodium concentration fell sharply, indicating movement of water out of cells and perhaps transfer of sodium into cells. Increased Na excretion could not account for the fall, since the concentration of Na in urine was well below that in serum. The fall in serum Na concentration was not sufficient to prevent some rise in osmotic pressure of serum. When these changes occurred, the excretion rate of Na increased 2- to 6-fold. In contrast to glucose and mannitol, urea, although excreted in comparable amounts, neither increased Na excretion nor significantly altered serum Na concentration. Increased Na excretion following glucose and mannitol infusions may be due to expansion of extracellular volume rather than to an osmotic effect in the renal tubules alone, since comparable urea infusions neither expand the extracellular volume nor augment Na excretion. The metabolism of K, though closely linked to cellular metabolism, is also affected by osmotic distortions of internal environment. Infusions of mannitol, glucose or urea had some effect on K excretion, producing slight initial increases during dehydration and definite decreases during water diuresis. Serum −K concentration was not significantly affected by expansion or contraction of extracellular volume. The single striking change in serum K occurred following glucose infusion during water ingestion when serum concentration fell by 25%, associated with transfer of 15 meq. of K into cells. This occurred when extracellular volume was contracting and glucose utilization was accelerated, and was probably related to glucose metabolism.

Graded arterial pressure decrement and renal clearance of PAH, creatinine, and sodium. EWALD E. SELKURT, PHILIP W. HALL (by invitation), and MERRILL P. SPENCER (by invitation). *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, Ohio.* When arterial pressure is gradually decreased by tourniquet action on the dorsal aorta just above the left renal artery in the dog, the clearance of creatinine decreases at first gradually from about 150 to 90 mm. Hg, then more rapidly to cease entirely at about 50 mm. Hg. The relationship of clearance to pressure is thus curvilinear, concave to the pressure axis. The clearance of PAH declines more slowly than that of creatinine, remaining near the control value until about 80 mm. Hg, then decreasing abruptly to extinction as glomerular filtration ceases and anuria ensues. When clearance of PAH is related to pressure it shows curvilinearity with even greater concavity than that of creatinine. The more rapid decline of creatinine clearance signifies a decrease in filtration fraction as pressure is decreased. Renal tubular reabsorption of sodium was found to remain essentially complete throughout the range of glomerular filtration rates studied. Nevertheless, even though urinary excretion of sodium was small, there was a definite tendency for it to decrease further as glomerular filtration was reduced, and at approximately 50% of the control filtration rates urinary excretion of sodium ceased almost entirely.

Histo-physiology of the adrenal cortex. HANS SELYE and HELEN STONE (by invitation). *Inst. de Médecine et de Chirurgie expérimentales, Univ. de Montréal, Montréal, Canada.* Experiments on the rat reveal that—in addition to mere stimulation or inhibition of secretion—the adrenal cortex can respond in a highly specific manner to certain

agents. Chronic treatment with lyophilized beef-anterior-pituitary tissue (LAP), and to a lesser extent with extracts of various necrotic tissues (e.g., Walker rat tumor, rat kidney), cause myeloid metaplasia of adrenal cortical cells, with the formation of both leucocyte and erythrocyte forming elements. Testoid compounds (e.g., methyl-testosterone) cause metaplasia of adreno-cortical into ordinary fat cells, exhibiting the typical 'signet ring' appearance and containing a single, large fat vacuole. Simultaneous treatment with LAP and methyl-testosterone cause hemopoiesis in a fat cell-containing stroma. This may result in the complete transformation of fasciculata and reticularis into typical bone-marrow tissue, while the glomerulosa remains intact. Even acute and intense overdosage with purified corticotrophin fails to cause such severe loss of adrenal cortical lipid granules as is produced by systemic stress during the alarm reaction. Indeed, pretreatment with corticotrophin inhibits the loss of cortical lipid granules which is normally produced by alarming stimuli or folliculoid hormones. Hence, in addition to increased corticotrophin secretion other factors must prevail during the alarm reaction to effect the usual intense discharge of cortical lipids. Experimental procedures will be discussed which permit the production in the adrenal cortex of specific structural changes having hitherto received little attention. The most important among these are the formation of intracellular colloid bodies, cytoplasmic 'chromatin-like' basophilic granules, fibrinoid degeneration of adreno-cortical cells, megakaryocyte-like giant cells and of "pseudolumina" with holocrine secretion of adreno-cortical cells. The functional significance of these lesions will be discussed.

The pattern of localization in the motor cortex of the rat. P. H. Settlage (by invitation), W. G. Bingham (by invitation), H. M. Suckle (by invitation), A. F. Borge (by invitation) and C. N. Woolsey. *Depts. of Anatomy, Surgery and Physiology, Univ. of Wis., Medical School, Madison, Wis.* The findings of Woolsey and LeMessurier (*Federation Proc.*, 1948), that the pattern of cutaneous representation in somatic *area I* (postcentral homologue) of the rat's cerebral cortex consists of "a somewhat distorted picture of a rat with its various parts related to one another in much the same manner as in the actual animal", have led us to make a detailed study of the pattern of localization in the motor cortex of this rodent. The motor area lies medial and rostral to the tactile cortex and corresponds approximately to Krieg's *areas 4, 6, 10* and *8*. The axial musculature from tail to neck is represented along the medial edge of the motor area in a caudo-rostral sequence. The limbs are represented more laterally, with their apices meeting the apices of the limbs of the tactile pattern. The motor area for the head fills the rostral part of the field and extends laterally to the rhinal sulcus. The whole motor pattern is essentially a mirror image of the 'postcentral' tactile pattern. Together the tactile and motor systems of this animal show a clear and simple plan of organization which may prove fundamental to an understanding of less diagramatic arrangements in other forms.

Polyphenoloxidase activity of blood serum. Bernard Shacter (introduced by Michael B. Shimkin). *Lab. of Experimental Oncology, Univ. of Calif. Medical School, San Francisco, Calif. and the National Cancer Inst., National Institutes of Health, U.S.P.H.S., Bethesda, Md.* The phenoloxidase activity of human blood serum was investigated in the Warburg manometer. In the presence of serum the oxidation of catechol is initially retarded and then accelerated beyond the rate of auto-oxidation of the substrate. Adrenaline is acted upon in a similar fashion. The activity is apparently enzymatic since it is completely abolished by cyanide and by heating the serum to 80° for one hour. The rate of catechol oxidation increases as the temperature is raised from 25° to 43° and as the *pH* is increased from 6.6 to 7.8. Under fixed conditions of *pH* and temperature the enzymatic activity is proportional to the amount of serum used. Tyrosine, p-cresol, and resorcinol remain unoxidized when added to serum, while the auto-oxidation of hydroquinone is retarded. p-Aminobenzoic acid and sulfanilamide in 0.1 M concentration inhibit the oxidase activity. The data indicate the presence in blood serum of a polyphenoloxidase, specific for ortho diphenols.

Electrical phenomena in the squid giant axon. Abraham M. Shanes. *Dept. of Physiology and Biophysics, Georgetown Univ. School of Medicine, Washington, D. C. and Marine Biological Laby., Woods Hole, Mass.* The effects of calcium, potassium, veratrine, yohimbine, procaine, cocaine and DDT, on 3 components of the action potential following single spikes, have been studied oscillographically after localized application to axons from Loligo pealii. One component, a damped high frequency oscillation present in all fibers and dying away usually in less than 10 milliseconds, is closely related to the very similar subthreshold response. The negative after-potential (NAP), present in 50% of the untreated axons, has a maximum amplitude of 100–200 microvolts and disappears exponentially within 200 milliseconds; with veratrinization all fibers exhibit NAP, but this declines in two exponential stages, viz., an initial rapid one terminating in about 100 milliseconds and a slower phase lasting about one second. A positive after-potential, evident only after yohimbine, reaches a maximum of less than 50 microvolts within 75–100 milliseconds and lasts about one second. Cocaine and

procaine (.01–.02%) reversibly depress the oscillations and NAP whereas veratrine and lowered potassium enhance them. Calcium acts on the oscillations as described by Arvanitaki; it depresses the NAP when veratrine is absent, but increases it in the latter's presence. Only veratrine altered the time constant of NAP decline. DDT was completely inert. The oscillations grow in veratrine but decline in yohimbine during repetitive stimulation. These observations, and others to be described later for crab and frog nerve, appear explainable largely in terms of potassium transport near the fiber surface as governed by electrochemical factors and a labile permeability and metabolism.

Production of adeno-carcinoma of breast in Wistar rats by the gastric instillation of methylcholanthrene. HARRY SHAY, ERNEST A. AEGERTER (by invitation), MARGOT GRUENSTEIN (by invitation) and S. A. KOMAROV. *Samuel S. Fels Research Inst. and the Dept. of Pathology, Temple Univ. School of Medicine, Philadelphia, Pa.* Wistar strain rats placed on Zucker's calcium-free diet are known to readily develop ulcerations of the antrum of the stomach (*J. Nutrition* 30 : 301, 1945). By instilling methylcholanthrene (melting point 180° corrected) in 0.5 cc. olive oil by stomach catheter (Shay and Gruenstein, *J. Lab. and Clin. Med.* 31 : 1384, 1946), we hoped to create suitable conditions for induction of carcinoma in the ulcerated stomach. Such experiments were performed on 5–7 weeks' old rats weighing 60–70 gm. and methylcholanthrene administered in 2 mg. doses (6 days a week) for 6–14 months. No gastro-intestinal tumors resulted but breast adeno-carcinoma developed in 15 out of 28 females. Calcium deficiency was apparently not essential for development of these tumors since they occurred in 4 out of 10 methylcholanthrene treated females on colony diet. The shortest period for development of tumors was 143 days and the longest 418 days. No tumors were observed in 12 similarly treated castrated females and 43 males, 24 of which were castrated. The high incidence of tumors induced distally by methylcholanthrene is significant, since in 15 years with an annual turnover of 2500 animals no breast adeno-carcinoma occurred spontaneously in our colony of Wistar strain rats. Keston (Conference on Ageing, Wistar Institute—June 1946) found no breast adeno-carcinoma in 630 autopsies on Wistar rats (mean age 810 days, S. D. 210 days), 164 of which had other tumors.

Isotopic studies of potassium exchange rates between cellular elements and plasma of human and canine blood *in vitro*. C. W. SHEPPARD and W. R. MARTIN (introduced by ALEXANDER HOLLAENDER). *Biology Division, Oak Ridge National Laby., Oak Ridge, Tenn.* Fresh heparinized blood is maintained in an apparently viable state for 8 to 10 hr. *in vitro*. Hemolysis is generally at or below the lower limit of visual detection. Hematocrits are routinely determined and lie within 2½% of the mean. There is some evidence of occasional systematic increases of potassium concentration in the plasma late in the experiments, the total change being about 15%. In human blood the concentration of isotope (K^{42}) in the plasma decreases fairly rapidly. Equal specific activities of cells and plasma are reached at about 18 hr. The curve is a smooth exponential with a single half value time corresponding to an exchange rate of about 10^{-13} millimol of potassium/hr/cell. In the dog the curve shows an initial rapid exponential decline to an elevated base line which slowly declines at about 1%/hr. Removal of a fraction of the cells containing leukocytes, platelets and probably young erythrocytes eliminates the rapid exponential phase. The slow decline is evidently characteristic of the exchange of potassium in mature canine erythrocytes, the rate being about 4×10^{-15} millimol/hr/cell.

A fluorometric method of determination of adrenalin in blood. WM. W. SHINGLETON and H. M. BAKER (introduced by K. S. GRIMSON). *Dept. of Surgery, Duke Univ., Durham, N. C.* Numerous chemical and biological methods planned for determination of adrenalin in blood have yielded variable results and failed to consistently determine small amounts of adrenalin which might be present in the circulating blood normally or during abnormal circumstances. Three reported chemical methods have been tested. The colorimetric method of Shaw, its modification by Raab, and the fluorescent method of Lehman and Michealis yielded results which were not consistent or capable of repetition. A new method has been developed employing the selective adsorption of adrenalin by $Al(OH)_3$ at *p*H 8.3 and a fluorescent determination of this adsorbed adrenalin. Fluoresence of the material oxidized by 25% NaOH was measured using a Coleman Electronic Photofluoremeter equipped with special excitation and transmission filters. This method was tested many times using freshly drawn heparinized human venous blood. Plasma obtained by centrifuging was divided into three equal samples. Known equal amounts of adrenalin chloride were added to two of the samples. One cc. of formaldehyde was then added to one of the two adrenalin treated samples. Twenty-four hrs. later fluorescence of each sample was determined. The fluorescence of the sample without adrenalin and that with adrenalin plus formaldehyde was approximately equal and low. Fluorescence of the third sample containing adrenalin alone showed a high end point approaching 90% of the fluorescence of a sample of water with the same quantity of adrenalin and similarly treated. Using this method, preliminary experi-

ments have been performed which indicate that relatively small amounts of adrenalin injected into dogs or rabbits are detectable.

Hepato-renal factors. XXVII. Biological fractionation of humoral VEM and VDM in renal hypertension (dogs). EPHRAIM SHORR and BENJAMIN W. ZWEIFACH. *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* The initial acute stage of experimental renal hypertension in dogs is associated with the appearance in blood of the renal vasoexcitor, VEM. During the subsequent chronic hypertensive stage, the blood progressively reverts to a neutral reaction, as judged by the rat mesoappendix test, presumably due to the presence of both VEM and the oppositely acting hepatic vasodepressor, VDM, in physiologically equivalent amounts. More detailed analyses have since been made of the sequence in which these vasotropic principles appear in the development of the hypertensive syndrome, utilizing procedures which permit the separate quantitation of each factor when both are present. Development of antisera to VDM (ferritin) provided a tool for selectively inactivating the VDM components of hypertensive blood, and unmasking VEM. Conversely, the ability of normal kidney slices to inactivate VEM aerobically, without affecting VDM, permitted quantitation of the VDM titer. Untreated blood samples, from dogs with renal hypertension (Goldblatt clamp) gave vasoexcitor (VEM) rat tests in the first few weeks after clamping, during the period of rising blood pressure; and thereafter were neutral. Utilizing these fractionation procedures, VDM was found to appear as early as 7–14 days after clamping, during the period of VEM predominance. VDM titers then rose progressively until they masked humoral VEM in the rat mesoappendix test. Thereafter, there was a shifting balance between VEM and VDM, with transitory predominance of each. In animals with fatal malignant hypertension, the terminal stage generally was associated with a shift in equilibrium, with progressive predominance of VEM.

Effect of hypothermia on cardiodynamics. WILLIAM J. SHRIBER (introduced by A. H. HEGNAUER). *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Dogs weighing 9–15 kg. were anesthetized by intravenous pentothal. The right auricle was catheterized through the right external jugular vein; and the left ventricle was catheterized via the left carotid artery. Intracardiac pressures were recorded with critically damped electrical strain gauge manometers together with simultaneous electrocardiograms. The animals were then cooled in 2–4°C. water baths. Serial photographic records were taken until death. Left ventricular systolic pressure remained fairly constant until rectal temperatures of 25–23°C. and then fell rapidly. Right auricular systolic pressure remained relatively constant to similar thermal levels and then rose rapidly. These sudden changes in pressure appeared to be correlated with the cessation of respiration. The fall in the left ventricular pressure probably occurred as a result of an inadequate pulmonary circulation and this in turn resulted in an inability to maintain arterial blood pressure. The sudden increase in right sided pressure was again probably attributable to pulmonary circulatory resistance which led to right heart failure. Conceivably, hypoxia of the cardiac muscle might be a factor in the failure of the heart.

Diet and the activity of the insulin-inactivating system of rat liver. BENJAMIN SIMKIN (by invitation), ROBERT H. BROH-KAHN and I. ARTHUR MIRSKY. *The May Institute for Medical Research of the Jewish Hospital, Cincinnati, Ohio.* Our previous investigations revealed a marked diminution of the content of the insulin-inactivating system (insulinase) per gram of rat liver during a 72-hr. fast and a restoration of the content of this system to normal values following a 48-hr. period of re-feeding a balanced diet. The present investigation is concerned with the influence of various types of food-stuffs on the restoration of insulinase activity during such re-feeding after a 3-day fast. Re-feeding a high (85%) carbohydrate diet restored the insulinase content to values above those obtained by re-feeding a balanced diet containing 50% carbohydrate. Re-feeding a high (85%) fat diet, on the other hand, restored the insulinase content to values significantly below those obtained by refeeding with the balanced or the high carbohydrate diet. Inasmuch as the diets were iso-caloric and the rats gained approximately equal weights during the period of their re-feeding, it seems probable that the administration of a high fat diet definitely diminishes the content of the insulin-inactivating system in the liver. In other experiments, rats were force-fed by tube with increasing amounts of high carbohydrate and high fat diets. The various results will be presented and their significance briefly discussed.

A quantitative comparison of Goldberger's augmented unipolar limb leads (aV) with Wilson's original V-leads. ERNST SIMONSON AND ANCEL KEYS. *Laby. of Physiological Hygiene, Univ. of Minnesota, Minneapolis, Minn.* Goldberger (Unipolar Lead Electrocardiography, Philadelphia, 1947) considers his two-terminal (aV) electrode to be identical with Wilson's three-terminal (V) 'unipolar' electrode except for a 50% larger amplitude, so that both electrodes may be used interchangeably. Among electrocardiographers preferences for the aV or the V-electrode vary but no quantitative comparison has been made. In 20 normal men and in 6 patients with abnormal axis deviation, the amplitudes of the QRS-complex and

the T-wave in the three limb leads were taken with Goldberger's two lead and Wilson's three lead terminal. In the arms leads the *mean* augmentation obtained with Goldberger's method agrees fairly well with the predicted 150% of the Wilson leads, but in lead aV/ the mean is 184 (QRS-complex) or 187% (T-wave). The finding in aV_F was confirmed in 22 other subjects. Moreover, in all leads the variability of augmentation between individuals is so great that prediction of the ratio aV/V for the individual is impossible. The results show that Wilson's three lead terminal and Goldberger's two lead terminal are not interchangeable, and that standards for one method are not applicable to the other method. The results have some bearing on the degree of validity of Einthoven's triangle hypothesis.

Diurnal variations in glomerular activity in normal man and in patients with congestive heart failure. JONAS H. SIROTA, DAVID S. BALDWIN and HERMAN VILLARREAL (introduced by HOMER W. SMITH). *Dept. of Physiology and Medicine, New York Univ. College of Medicine, New York City.* Renal clearances (4- to 6-hr. periods) of insulin, p-aminohippurate and endogenous creatinine were determined throughout 24 hr. in 13 normal males and in 5 patients with congestive heart failure with persistant edema. Test substances were administered by a constant delivery pump through a plastic intravenous catheter. Urine was collected by spontaneous voiding in normals and by catheterization in heart failure patients. Blood was drawn at the midpoint of each period. With few exceptions the subjects were at bed rest for at least 24 hr. prior to as well as during the period of observation and slept approximately from 8 P.M. to 6 A.M. No change in the dietary regimen was made during the test day. Waking clearances were compared with those during sleep and expressed as night to day ratios (N/D). In the normals the average N/D ratio for C_{IN} was 0.94, for C_{PAH}, 0.97, and for endogenous creatinine clearances, 0.97. In patients with heart failure the average N/D ratio for C_{IN} was 1.18, for C_{PAH}, 1.14, and for endogenous creatinine clearances, 1.16. The endogenous creatinine/insulin clearance ratio averaged 0.92 in the normals, and 0.75 in patients with heart failure. It is concluded that glomerular activity is typically unchanged or decreased during sleep in normal subjects, whereas it typically increases during sleep by a significant amount in heart failure. The endogenous creatinine clearance is not a reliable index of filtration rate in subjects with heart failure.

Augmentation by catechol and p-cresol of the oxidation of proteins by mushroom tyrosinase. IRWIN W. SIZER and CLYDE O. BRINDLEY (by invitation). *Dept. of Biology, Massachusetts Institute of Technology, Cambridge, Mass.* In previous experiments we have shown that certain proteins can be oxidized by tyrosinase and, in the case of invertase, this oxidation is accompanied by a decrease in invertase activity. Since the oxidation of certain substrates by tyrosinase is accelerated by the addition of an activator (See Nelson, J. M. and Dawson, C. R., *Adv. in Enzymol.* 4: 99, 1944) we have tested such activators on the tyrosinase protein systems. As little as 1×10^{-4} M catechol or p-cresol greatly accelerates and renders more extensive the action of tyrosinase on certain proteins. In the action of tyrosinase on invertase the phenolic activator seems to be coupled to the system like a co-enzyme. The reduced and oxidized form of the phenolic activator constitute a reversible oxidation-reduction system; i.e., catechol $\rightleftarrows$ o-benzo-quinone (see Nelson and Dawson). A side reaction is the slow formation of quinone polymers from the oxidized forms of the phenolic activators. These intermediary and final oxidation products of catechol had no effect on invertase activity in the absence of tyrosinase. Insulin oxidation by tyrosinase is greatly accelerated by the presence of traces of catechol, as indicated by a decrease in the ultraviolet absorbtion spectrum and a decrease in biological activity. Similar results are obtained with crystalline pepsin where tyrosinase in the presence of catechol produced a marked change in the ultraviolet absorbtion spectrum of pepsin and a decrease in peptic activity. Indications have been obtained that tyrosinase in the presence of certain phenolic compounds will oxidize many other proteins containing a high percentage of tyrosyl groups.

Comparison of transmission spectra of blood in the human ear flushed by histamine with those obtained after heat flushing. WILLIAM SLEATOR, JR., JAMES O. ELAM, D. J. KILIAN, W. N. ELAM, JR. (introduced by H. L. WHITE). *Dept. of Physiology and Dept. of Surgery, Washington Univ. School of Medicine, St. Louis, Mo.* Using the monochromator and amplifier arrangement already described (Neville *et al.*, *Federation Proc.* 1949), the percentage transmission of the ear lobe or pinna of a number of normal subjects was measured as a function of wave length from 5000Å to 11,000 Å. Curves were taken with the subjects breathing air, 100% O_2, and 12% O_2; and with the ears flushed by means of histamine administered electrophoretically, or by means of heat (which has been the principal arterializing agent used in oximetry). For both heat and histamine the percentage transmission when the subject breathed pure oxygen was, on the average for λ less than 7800 Å, about 15 to 20% higher than when the subject breathed 12% O_2; for λ greater than 7800 Å the 100% O_2 curve was 3 to 10% lower than the 12% O_2 curve. With histamine the spectrum when the subject breathed room air followed the 100% O_2 spectrum

closely through the whole wave length range, rarely differing by as much as 4%. This would be expected if the ear were really arterialized since normal arterial O_2 saturation is known to be above 95% on air and 99–100% on pure O_2. With heat, however, the room air curves were markedly (10–20%) below the 100% O_2 curves, but followed the 12% O_2 curves very closely (3–6%). The presence of a significant fraction of venous blood (O_2 saturation 75–80%) in the heated ear would account both for the large difference between 100% O_2 and air, and the small difference between air and 12% O_2.

Peripheral vascular reactions in the wing of the bat after total-body X irradiation. Douglas E. Smith, George Svihla (by invitation) and Harvey M. Patt. *Biology Division, Argonne National Laby., Chicago, Ill.* A number of peripheral vascular reactions (erythema, hyperemia, increased capillary fragility, hemorrhage) have been described in man and laboratory animals following exposure to X radiation. In the hope of obtaining a definitive picture of the development of these phenomena microscopic observations and cinema microphotographs were made of vascular beds in the wing of the living, intact bat (*Myotis lucifugus*) before and at various intervals after total-body X irradiation. Studies were made on several hundred animals during the late autumn and winter. Until high dosages (10,000–60,000 r) were reached no peripheral vascular changes were observed. Within an hr. after such dosages large numbers of leucocytes, many of which stuck to the vessel walls, were apparent. This phenomenon which was most marked during the first 24 hr. after irradiation persisted for several days. Early after irradiation the blood seemed to be less viscous than normal only to become more viscous at the end of 24 to 36 hr. and remain so until death. Consistent changes in vessel diameter, blood flow and venomotion were not observed, nor were any indications of hemorrhage. Toxicity experiments suggested that the bat might be highly resistant to X irradiation, for in these experiments life span was shortened only after dosages of 15,000–60,000 r. Significantly, only such high dosages produced the peripheral vascular changes noted above.

Observations of the action of the cardiac, pulmonic and aortic valves of the beating heart (motion picture). H. L. Smith (by invitation), Hiram E. Essex, and E. J. Baldes. *The Mayo Clinic and Mayo Foundation, Rochester, Minn.* Perfusion of the isolated heart of the rabbit or dog makes possible direct observation of the action of the cardiac, aortic and pulmonic valves. Observation of the mitral and tricuspid valves is facilitated by incising and retracting the atrial walls. With proper illumination, the pulmonic valves can be observed by cutting and retracting the walls of the common pulmonary artery. The observation of the aortic valves requires a special cannula and an apparatus for providing peripheral resistance to the flow from the left ventricle. Certain observations are discussed.

Effect of salt-sugar solution and DCA on lactation. Thomas C. Smith (introduced by F. L. Hisaw). *The Biological Labys., Harvard Univ., Cambridge, Mass.* Four-month-old rats were adrenalectomized and maintained with 1% NaCl, 1% glucose in the drinking water. Three days after adrenalectomy, they were paired with normal males. At parturition, some were continued on salt and glucose, while others were given tap water to drink and injected with DCA in .2 ml. sesame oil daily during lactation. The ability to lactate was measured by the growth and survival of the litter, reduced to 4 young; the growth of comparable litters of normal mothers served as controls. Salt and sugar therapy enabled adrenalectomized mothers to maintain normal pregnancies and undergo normal parturition, but in no case was lactation normal. Similarly, lactation was not normal in animals given 0.1 or 0.5 mg. DCA daily. One mg. DCA resulted in significantly improved lactation, with all litters, though underweight, surviving until weaning. Two mg. DCA gave normal lactation. Nearly normal body weight and carbohydrate stores have been maintained in adrenalectomized rats with 1% salt solution or 0.5 mg. DCA (Anderson and Herring, *Proc. Soc. Exp. Biol. & Med.* 43: 363, 1940). The present results show that salt and sugar or DCA (0.1, 0.5 mg.) will not maintain lactation but larger doses of DCA (1.0, 2.0 mg.) are effective.

Rate of elimination of sulfadiazine from the blood in the newborn. Franklin F. Snyder. *Depts. of Obstetrics and Anatomy, Harvard Medical School, Boston, Mass.* Sulfadiazine was found in the blood for a prolonged period in premature, newborn, and early suckling infant rabbits in contrast to adults, following subcutaneous injection of a standard dose, 0.5 gm/kg. Thus, the blood was negative in adults in less than 12 hr., while in premature animals of 30 days the blood contained 6 mg/100 cc. 24 hr. after injection. At 18 hr., the blood level in full term animals of 32 days was 14 mg.; in suckling infants of 1 week it was 5 mg.; and at 2 weeks or older the blood was negative. Much higher concentration of sulfadiazine was noted in the blood of the newborn than in that of adults despite uniform dosage. At one hour following injection, the blood level in adults averaged 22 mg. while in prematures it was twice as great, averaging 47 mg.; in the newborn at term it was 41 mg.; in suckling animals at one week it was 33 mg., and at 4 weeks was 27 mg. At 6 hr. after injection, the blood level in the newborn averaged 35 mg. in contrast to 7 mg. in adults. In all samples

obtained from 150 rabbits, determinations were made before and after hydrolysis using Marshall's method. The foregoing results are of particular interest in connection with the clinical observation of increased hazard for the child associated with sulfadiazine therapy at the time of birth or preceding it.

Ratio between tension and total electrical activity in human skeletal muscle. R. SNYDER (by invitation), C. F. S. FISHER (by invitation), and A C. BURTON. *Dept. of Biophysics, Univ. of Western Ontario, London, Ontario, Canada.* Most electromyography has used needle electrodes and recorded action currents of local single muscle units. General opinion has been that since different units were out of phase, the total integrated electrical activity, recorded from surface electrodes at the end of the muscle, would not correlate well with total tension. This is an error in statistical theory, and we find, using the flexor muscles of the arm and rectifying the output of an electromyograph, that there is a reproducible relation between tension and output. With the upper arm vertical, the elbow supported, and forearm horizontal, increasing loads are hung on the wrist. The output increases with increasing load, with a tendency for the slope of the graph to increase at high loads. Resistance of electrodes is not a disturbing factor. With a constant load, the output remains constant until fatigue sets in, when the electrical activity steadily rises. The ratio of tension to electrical output (T/E) is very different in the flexor arm muscles of different individuals, being much greater where the muscle is larger and presumably 'trained'. The measurement of the ratio offers a way of following the development of muscles by training and after disease, and of studying the process of fatigue. A consistent theory would be that in training and in fatigue, the electrical output of the single units is relatively constant, while the tension exerted is variable.

Pressure responses of the cerebrospinal fluid to diffusion respiration and carbon dioxide inhalation. JOSEPH N. SPENCER (by invitation), ELI S. GOLDENSOHN (by invitation), RICHARD W. WHITEHEAD, ROBERT F. GROVER (by invitation) and WILLIAM B. DRAPER. *Dept. of Physiology and Pharmacology, Univ. of Colorado Medical Center, Denver, Colo.* Simultaneous observations on cerebrospinal fluid pressure, systemic arterial and venous blood pressures, and heart and respiratory rates were recorded continually from dogs by means of strain gauge manometers. Venous blood *p*H was determined at intervals. Observations were made during diffusion respiration and with various concentrations of CO_2 in O_2 administered by inhalation under light thiopental sodium anesthesia. With diffusion respiration and the various concentrations of carbon dioxide administered, the cerebrospinal fluid pressure was seen to rise consistently to at least twice the control value, and to an average of 3½ times the control value. The maximum elevation is frequently observed within 10 minutes. During diffusion respiration, cerebrospinal fluid pressure rose slowly to 3½ times the control value after about 15 minutes. The pressure then declined from its peak but remained elevated. During inhalation of 40% CO_2 in O_2, the maximum rise in cerebrospinal fluid pressure was usually reached in 5 minutes and rose to 4½ times the control pressure. Changes in heart rate, respiration, and arterial pulse pressure were reflected in the cerebrospinal fluid pulse waves. However, neither these factors nor variations in arterial blood pressure showed close correlation with the observed rise in cerebrospinal fluid pressure. Studies on the effects of inhalation of 10, 20 and 30% CO_2 in O_2 will be reported.

Spectral deviations found in hemolyzed erythrocytes of rabbits fed a purified diet. S. S. SPICER, J. G. WOOLEY, and ARIEL M. CLARK (introduced by WILLIAM J. BOWEN). *Experimental Biology and Medicine Inst., National Institutes of Health, Bethesda, Md.* Rabbits maintained on a purified diet containing leached alcohol extracted casein, sucrose, Wesson oil, cellophane, minerals and pure vitamines have survived for a period of months to years. Some of these animals have shown an unusually dark color in the suspension of their washed red cells. Below 5800 Å, the absorption spectra of hemolysates from purified diet rabbits appeared normal. However, the 1/20 dilution of washed red cells of these rabbits in saponin buffer revealed abnormally high absorption between about 5900 and 6500 Å. This absorption was evident in the oxygenated hemolysate whether buffer of *p*H 6.2, 7.9, or 9.0 was used. Subtracting the mean molar (equivalent) extinction coefficient of hemolysates (*p*H 7.4) from several stock diet rabbits from that of the purified diet animals showed a maximum at 6200 Å in the oxygenated and 6150 Å in the CO treated solution. The optical density of the CO derivative exceeded that of the oxy form by about 40%. In hemolysates of the rabbits fed purified diet, when the hemoglobin was converted to methemoglobin at *p*H 8.6 or to metcyanhemoglobin there was slightly increased density occurring uniformly without the presence of an absorption band between 5800 and 6500 A. The band in the oxygenated hemolysate was noted spectroscopically in 6 of 16 animals maintained on purified diet and in none of 15 rabbits on the laboratory stock diet for 1 to 3 years.

Electroshock studies: (a) Convulsant effect of microwaves. (b) Changes of current during stimulation. E. A. SPIEGEL, P. H. WILCOX (by invitation), and A. J. LEE (by invitation). *Dept. of Exper. Neurology, Temple Univ. School of Medicine,*

Philadelphia, Pa., and Traverse City State Hospital, Traverse City, Mich. a) The convulsant effects of repeated rectangular microwaves were studied in guinea pigs using a generator with pulses of 5 to 40 microseconds at frequencies from 60 to 800 per second and Offner's brief stimulus apparatus with pulses from 50 microseconds upwards at frequencies of 60 and 120 per second. The electrodes were applied bitemporally on the intact skull. Generalized epileptiform convulsions outlasting the stimulating current could be produced at a minimum duration of the individual pulses of 10 microseconds. Quantity-duration curves for the component pulses (abscissa: msec.; ordinate: microcoulombs) obtained with Offner's stimulator (120/sec.) showed that the necessary pulse Q's decrease as the total duration of the stimulation is increased from 0.1 second to 1.0 second and also as the duration of each pulse is decreased from 0.5 msec. to about 0.05 msec. Temporal summation at this frequency apparently is sufficient to reach the convulsive threshold even when relatively few neurones are stimulated with each pulse, thus decreasing the possibility of supramaximal stimulation of some of the neurones.

(b) Cathoderay oscillograph records of voltage and current during stimulations with 60 cycle A.C. or halfwave rectified A.C. revealed a definite (up to 40%), regionally unspecific increase of current within the first 0.1 second. The decrease of resistance appears also on subthreshold stimulation. It is still marked 10 minutes after cessation of stimulation. The resistance to a large extent depends on the voltage applied; it is considerably lower with high than with low voltage.

Hemorrhagic diathesis in leukemia. MARYLOO SPOONER (introduced by W. J. MEEK). *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison, Wis.* Few studies concerning the cause of the hemorrhagic tendency in leukemia have appeared in the literature. A group of tests including coagulation time, clot retraction, prothrombin time, antithrombin titer, bleeding time, capillary fragility test, vitamin C levels of blood and plasma, and fluorescein test for capillary permeability was run on 33 patients with clinical diagnoses of various types of leukemia. Patients with Hodgkin's disease and lympho sarcoma were used as controls making a total of 50 patients. An attempt was made to correlate the findings with the presence of hemorrhagic signs. Results show that a correlation does not necessarily exist between positive laboratory data and the presence of hemorrhagic signs. In the group of patients with acute leukemia, there was a better correlation between positive laboratory data and clinical evidence of hemorrhage. Increased capillary fragility did not correlate with increased capillary permeability as determined by the fluorescein test. Lower vitamin-C levels were found in the control group, which rarely showed hemorrhagic signs, than in the leukemic group which had a higher incidence of hemorrhagic signs. The data do not indicate whether or not hemorrhages might occur at some future time. As in other laboratory tests, a series of the same determination or a group of different determinations will yield more information than a single test.

Changes in blood pressure and renal function in hypertensive dogs following abscess production. J. STAMLER (by invitation), S. RODBARD and L. N. KATZ. *Cardiovascular Dept., Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* The production of an abscess in hypertensive dogs leads to a fall in blood pressure which usually begins within 24–48 hr. of the tissue injury and persists for periods up to 3 weeks. This fall in blood pressure was studied in 9 dogs; it occurred when either subcutaneous tissue implantation or intramuscular carbon tetrachloride injection was used to incite abscess. As part of an extensive project investigating the mechanism of this phenomenon, renal clearance studies were done on these dogs prior to and following abscess production. These studies showed that abscess formation is also associated with a significant increase in renal blood flow, which usually begins within 24–48 hr. and persists for as long as three weeks. This was more marked and prolonged in those dogs showing a greater, more sustained blood pressure fall. The increase in renal blood flow was not always synchronous with the fall in blood pressure. No significant changes in glomerular filtration rate were noted; the filtration fraction was decreased; renal vascular resistance was significantly lowered. The data suggest that the fall in blood pressure after abscess production may not be causally related to the increased renal blood flow (relief of renal ischemia) observed in these hypertensive dogs.

Further direct experiments on the relation between ballistocardiograms and systolic velocity curves. ISAAC STARR, R. L. MAYOCK (by invitation) and O. HORWITZ (by invitation). *Depts. of Therapeutic Research and Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* We have continued the experiments of the type reported at the last meeting. Using cadavera lying on the ballistocardiograph at necropsy, fluid has been injected into the aorta alone, the pulmonary artery alone, and into both together. The amount of the injection was optically recorded at each instant. Aortic blood pressure was secured by a Hamilton manometer in the later experiments, and a normal diastolic pressure was attained by connecting a pressure bottle with a femoral artery. 'Normal' injections produced normal pressure pulse waves. We have secured good records of about 100 'systoles' in 6 cadavera. The deflection of the ballistocardiogram

is caused by the acceleration of the injected fluid and not by its velocity per se. The contour of the ballistic record follows that of the calculated curve of acceleration, lagging a little behind it. The 'J'-wave follows the contour of the first part of the acceleration curve with a fidelity which has surprised us; the latter part of the acceleration curve is not so accurately reflected in the ballistocardiogram unless the initial impacts are very small. Evidently after-vibrations from large initial impacts distort the latter part of the curve in more 'normal' records. In these experiments the 'I'-wave was smaller than is found during life, probably because the shape of the cannulae used did not reproduce the movement of blood within the heart during systole. For a given acceleration fluid injected into the pulmonary artery causes less initial deflection of the ballistocardiogram than fluid injected into the aorta.

Interrelation of effects of intraarterial injections of histamine and acetylcholine on gastric secretion. GEORGE W. STAVRAKY. *Dept. of Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.* As previously reported, acetylcholine injected into a quiescent stomach through the gastrosplenic artery causes an alkaline secretion which is free from pepsin (G. M. Morton and G. W. Stavraky, *Federation Proc.* 7: 82, 1948). Similar intraarterial injections of histamine in 1/300,000 dilution (100–200 cc/hr.—17 kg. dog) are found to evoke a secretion of highly acid gastric juice of low digestive power. More concentrated solutions of histamine inhibit the secretion of hydrochloric acid. Addition of acetylcholine (1/100,000 concentration) to the solution of histamine lowers the acidity of the gastric juice but markedly activates the secretion of pepsin. Also, if acetylcholine is injected before histamine (causing an alkaline secretion without pepsin) a subsequent injection of histamine evokes a reduced secretion of acid but the gastric juice secreted in response to histamine now contains large quantities of pepsin. When atropine is injected before a combined administration of acetylcholine and histamine, it precludes the secretion of pepsin. However, interposed between the injections of acetylcholine and histamine, atropine does not prevent histamine from causing a secretion of gastric juice of high digestive power despite the fact that before atropinization acetylcholine caused a secretion which contained no pepsin. The results of these experiments seem to warrant the conclusion that a preexisting or combined cholinergic stimulation of the glandular elements of the gastric mucosa is necessary in order that histamine may cause a secretion of pepsin.

Pressure and activity recordings in the pylorus and rectum of man. F. R. STEGGERDA and W. C. CLARK (by invitation). *Dept. of Physiology, Univ. of Illinois, Urbana, Ill.* By means of a pressure recording pick-up, consisting of a small water capsule at the end of a rubber catheter connected to an electronic amplifier, a continuous record of pressure changes can be made in various locations along the gastro-intestinal tract. In preliminary observations it was noted that there were very obvious differences in pressure and activity when the water capsule was believed to be passing from the stomach through the Pyloric Sphincter region and on into the small intestine. That these changes in activity and pressure recordings are consistent has been verified in experiments on 4 different individuals. Furthermore that this change in activity and pressure is found only in the Pyloric Sphincter region is confirmed by X-ray examinations. The actual pressure in the Pyloric region was 5–10 cm. of water higher than on either the stomach or intestinal side of the sphincter area and the activity showed a tonic rhythm of 11 contractions per minute, an observation very different from that recorded in the stomach or intestine. When comparative studies of pressure and activity were made between the rectum and sigmoid colon, differences were also recorded.

Effect of coffee on the growth of white rats. ARTHUR H. STEINHAUS and AARON SPITZER (by invitation). *George Williams College Laby. for Physiologic Research in Physical Education, Chicago, Ill.* A coffee infusion interfered markedly with the growth of white rats when this was added to a milk diet fortified with iron and copper. Rats fed the fortified milk plus coffee infusion from the time of weaning averaged a gain of 49 gm. in body weight in 9 weeks. Litter mates consuming identical quantities of milk plus tap water or milk plus .5% tannic acid in water, both equivalent to volume of coffee infusion, averaged a gain of 87 gm. in the same period. When the diets of the 'coffee rats' and 'tannic acid' rats were switched at about 13 weeks of age, the direction of the growth curves was modified as expected, but not to as great an extent. This observation exonerates the tannic acid in coffee as the growth interfering factor. Studies now underway are designed to determine more accurately the 'coffee effect' at different ages and whether the observed growth failure is due *1)* to the diversion of food to sustain a greater amount of spontaneous activity or *2)* to some interference with its utilization.

Metabolism of pigeons in extreme cold. E. STREICHER (by invitation), D. B. HACKEL (by invitation), W. FLEISCHMANN and G. L. BULGER (by invitation). *Physiology Section, Medical Division, Army Chemical Center, Md.* The ability of fasting pigeons to survive up to 78 hr. in extreme cold (−35°C.) has been demonstrated (Horvath *et al. Science* 107: 171, 1948). Further studies have been carried out to elucidate the metabolic factors

involved in this long survival time. The metabolic rates of pigeons weighing between 275 and 625 gm. have been studied by means of an apparatus consisting of a desiccator jar of known volume containing a CO_2 absorber. The metabolic rate is estimated simply by determining the change in O_2 content of this chamber. At room temperature (24°C.) the range for the basal metabolic rate is from 105–125 C/kg/24 hr. This rises to about 375 C/kg/24 hrs. after 4 hr. at −40°C., and varies between 300 and 500 C/kg/24 hr. until the terminal drop in body temperature. Blood glucose remains within the normal range up to 72 hr., although the liver glycogen is almost depleted by the end of 24 hr.

Effect of cold adaptation on highest and lowest temperatures at which turtle heart continues beating. D. E. STULLKEN (by invitation), F M. WHITE (by invitation) and W. A. HIESTAND. *Laby. of Animal Physiology, Purdue Univ., Lafayette, Ind.* Turtles were kept at 4°C. as long as 10 weeks to allow 'cold adaptation'. The hearts of these turtles were placed in Ringer solution with 'control' hearts of non-cold adapted turtles. The temperature of the Ringer bath was slowly lowered and the temperatures at which the beating ceased was recorded. After both hearts had stopped beating at the lower of the two temperatures the bath was then heated and the temperature raised slowly until both hearts resumed beating and continued until each stopped at its respective high temperature. The results were as follows: With falling temperature the cold adapted hearts stopped beating at an average temperature of 11.3°C. (av. of 8 hearts) while the non-cold adapted hearts stopped at an average temperature of 1.03°C. (av. of 8 hearts). With rising temperatures the cold adapted hearts stopped at an average temperature of 38.9°C. (av. of same 8 hearts) while the non-cold adapted hearts failed at 36.2°C. (av. of same 8 hearts). Evidence shown by the cardiograms indicates that the sinus tissue is apparently affected by cold adaptation more than the myocardium. It is surprising that the cold adapted heart failed at a higher temperature than did the non-cold adapted heart with falling temperatures. Also paradoxically the cold adapted heart continued beating at a higher temperature than did the non-cold adapted heart with rising temperatures.

Centers governing temperature selection and the frequency response to temperature in the brain of trout. CHARLOTTE SULLIVAN (by invitation) and KENNETH C. FISHER. *Univ. of Toronto, Toronto, Canada.* Trout in a gradient of temperature normally remain at, that is, select, a comparatively narrow range of the temperatures available. This response to temperature disappears when lesions are made in the forebrain of these organisms but not when the dorsal portion of the cerebellum is destroyed. The frequency with which spontaneous movements occur in trout changes with temperature in a complex but reproducible manner. The general nature of this response is unaffected by damage to the forebrain but may be completely changed by destroying the dorsal part of the cerebellum.

Effect of posterior pituitary extract on man. F. SUNAHARA (by invitation), D. DUNCANSON (by invitation) and O. G. Edholm. *Dept. of Physiology, Univ. of Western Ontario, London, Canada.* Forearm blood flow was measured before and after the intravenous injection of 4 units of histamine-free posterior pituitary extract into healthy male volunteers. Measurement of cutaneous blood volume was made with a Millikan oximeter ear piece. Immediately following the injection, the subjects' faces become ashen in color, the pallor persists for approximately 60 minutes. The oximeter readings on the ear showed a definite diminution in ear blood volume, but these readings returned to normal before normal color returned to the face. The forearm flow shows a slight increase immediately after the injection and thereafter is within normal limits. The subjects complained of nausea and abdominal discomfort and a desire to defecate. In several cases there was slight headache. These symptoms and the appearance of the subject are strikingly similar to those observed after fainting as a result of haemorrhage.

Effects of low environmental atmospheric pressure on the blood of turtles. LOUIS A. SUSCA (introduced by CHARLES G. WILBER). *Biological Laboratory, Fordham University, New York City.* Susca and Wilber (1948) reported changes in the cholesterol content of the blood of turtles of the variety *Pseudemys* sp. after subjecting them to pressures equivalent to various altitudes. Further pressure studies indicate that changes occur also in the glucose and phospholipid content of the blood of turtles. Estimations for glucose in the blood, using the Somogyi filtrate, were made for each of 10 untreated animals, and also, for 10 turtles subjected individually to pressures equivalent to altitudes of 18,000; 33,000; 48,000; 64,000; and 90,000 ft. respectively. Estimations for the phospholipid content of the blood of turtles similarly treated also were made. The accompanying ta-

	CHOLESTEROL (PREVIOUS REPORT)	GLUCOSE	PHOSPHOLIPID
Normal	38.2	51.8	14.4
18,000	108.5	110.2	14.0
33,000	118.5	129.4	21.8
48,000	87.2	219.6	12.6
64,000	70.1	188.2	50.9
90,000	49.2	205.6	9.1

ble gives the mean value, in mg/100 cc. of blood, for each of the substances estimated. Each value represents the mean of 10 readings, each of which is for the blood of a different animal.

Considerable variation occurs at different pressures, but changes within a single group of 10 animals are of the same order of magnitude. Emphasis in the changes is noted at pressures equivalent to altitudes of 33,000 and 48,000 ft. respectively.

Effects of various concentrations of potassium cyanide on *Pelomyxa carolinensis*. LOUIS A. SUSCA (by invitation) and CHARLES G. WILBER. *Biological Laby., Fordham Univ., New York City.* A detailed study of the effects of .001M; .005M and .05M KCN on *Pelomyxa carolinensis* indicates that the cellular structures of the amoeboid organism are affected. Changes in the number, size, and shape of nuclei occur after the organisms are placed in the various solutions for more than 24 hr. In a .001M solution, the large number of nuclei characteristic of the organism is not affected; in a .005M solution, very few nuclei appear; in a .05M solution, a very small number of nuclei is observed. Sizes of the nuclei vary from 12 micra to 14 micra in diameter in organisms placed in .05M KCN, to 24 micra to 28 micra in organisms placed in .001M KCN. In .001M KCN, nuclei are spherical or elongated in shape; in .005M KCN, nuclei are ellipsoidal in shape; in .05M KCN, nuclei are either spherical or elongated in shape. The number of contractile vacuoles is largest in organisms placed in .05M KCN. Many contractile vacuoles are observed in organisms placed in .001M KCN, and very few are noted in organisms placed in .005M KCN. The distribution and spherical shape of refractive bodies in the organisms are not affected by any of the solutions. Food vacuoles form at frequent intervals in organisms from each solution, but fewest are formed in organisms placed in .05M KCN. The bipyramidal and truncated crystals are 3 micra in width in organisms placed in .001M KCN; mostly 1.5 micra in width in .005M KCN; and 1.5 micra to 3 micra in width in .05M KCN.

Free amino acids in the plasma of Eck fistula, meat intoxicated, and hepatectomized dogs. MURIEL H. SVEC (introduced by SMITH FREEMAN). *Dept. of Experimental Medicine, Northwestern Medical School, Chicago, Ill.* Sixteen free amino acids were measured in the plasma of fasting dogs by microbiological assays. Alanine and glycine were determined chemically. Normal ranges were established on series of 12 to 36 dogs for each of 13 amino acids. Following Eck fistula formation, 4 of5 dogs showed an average reduction of 10μ/g. of free leucine/ml. of plasma. This decreased value remained within the previously established normal range. Twelve other amino acids showed no consistent changes. Five of 6 meat-intoxicated Eck fistula dogs showed definitely increased plasma histidine and arginine; 8 of 9 showed increased methionine and tyrosine. Five other meat-intoxicated Eck fistula dogs showed slightly elevated cystine and leucine levels. The remaining 6 amino acids studied varied inconsistently or not at all. Two normal dogs and 7 or 8 portal-obstructed dogs on the same horse meat diet did not show these changes. In a series of 15 dogs, during an 8 to 16 hr. period following hepatectomy, plasma methionine dropped markedly in 12, sometimes disappearing completely. These animals also showed a definite elevation of the histidine level. Tryptophane decreased in all of 8 dogs studied. Three out of 4 animals showed increased alanine and lysine. Determinations of glutamic acid in 5 dogs and glycine in 4 showed definite increases in plasma levels postoperatively in all animals. These changes were distinct from those of the control period during which the animals to be hepatectomized received by vein the amount of glucose to be given postoperatively.

Castration and the mobilization of nitrogen during fasting. CLARA M. SZEGO and ABRAHAM WHITE. *Dept. of Physiological Chemistry, School of Medicine, Univ. of California at Los Angeles, Los Angeles, Calif.* The mobilization of nitrogen from lymphoid tissue, liver and carcass of castrate male and female mice of the CBA strain was studied during a 48-hr. fast. Castration in both sexes inhibited the decline in the ratio of lymphoid tissue to body weight which normally accompanies the stress of total inanition. Loss of nitrogen from lymphoid tissue was concomitantly depressed. The composition of the liver tissue of fed castrate mice differed significantly from that of intact controls. Higher concentrations of liver lipids and greater liver weights were observed in the gonadectomized animals. Utilization of liver nitrogen during subsequent fasting, however, was unaffected by castration. The mechanism by which castration inhibits the fasting involution of lymphoid tissue is being investigated in relation to adrenocorticotrophic and gonadotrophic activity of the adenohypophysis.

Measurement of renal plasma flow in man by the slope method without urine analysis. HALL S. TACKET (introduced by C. RILEY HOUCK). *Divisions of Medicine and Physiology, Univ. of Tennessee, Memphis, Tenn.* The rate of disappearance from the plasma of a substance, previously administered by a single intravenous injection, represents the amount of that substance removed by the kidney per unit time, assuming no extra-renal loss. That rate, expressed as a fraction (S) of the total substance available for excretion, multiplied by the volume in which the total substance is distributed (Vc), gives the volume of fluid that contains the amount removed by the kidney per unit time, or the renal clearance (C). Thus: $C = VcS$.

We have determined para-aminohippurate clearance by this method in 12 experiments on 10 patients with no evidence of renal impairment, comparing results with simultaneously measured urine clearances. (Before colorimetric analysis, all plasma filtrates and diluted urines were hydrolyzed in 0.4N HCl at 97°F. for 3½ hours to free PAH conjugated by the liver.) Slope clearance based on the volume of distribution of PAH gave values that were consistently too high. However, using empirically the volume of distribution of mannitol, the quotient of the slope clearance and the urine clearance averaged 0.91 with a range from 0.65 to 1.08. In 8 of the 12 experiments, the quotient averaged 1.005 with a range 0.95 to 1.08. The reason for the failure to obtain completely consistent results is not yet evident, but good correlation was observed more frequently than can be explained fortuitously. We failed to find satisfactory correlation between the slope clearance and the urine clearance of mannitol. The explanation for this discrepancy, likewise, is not yet apparent.

Reabsorption of potassium by the kidney. ROBERT TARAIL and DONALD W. SELDIN (introduced by DONALD H. BARRON). *Dept. of Internal Medicine, Yale Univ. School of Medicine, New Haven, Conn.* From a survey of the literature one may surmise that a ratio of the concentrations of urine potassium to serum potassium below one has not been reported. Concentration ratios (U/P) below one would signify formation of renal tubular reabsorbate with a concentration of potassium greater than that of extracellular fluid. Such concentration ratios were observed during the following conditions: water diuresis; glucose diuresis during ingestion of water; and during recovery from the shock of 'dolophine' intoxication. When urine flows were 948 and 1008 cc/hour during water diuresis, in 2 normal subjects, urine potassium was 2.5 and 2.8 mEq/l. per liter, and concentration ratios were both 0.68. During glucose diuresis and water ingestion, urine potassium was 1.3 mEq/l., concentration ratio was 0.38, and urine flow was 1462 cc/hour. Such high rates of urine flow are not a necessary prerequisite for a concentration ratio below one. A ratio of 0.84 and urine potassium of 3.3 mEq/l. when the urine flow was only 135 cc/hour occurred in a patient sustained on fluids without potassium during recovery from peripheral vascular collapse. Despite the capacity of the kidney to produce a urine with a concentration ratio below one, this is rarely found during the development of potassium deficiency in man. Conditions affecting efficient reabsorption of potassium during low rates of urine formation have not been clearly defined, but probably include the state of hydration and metabolic factors.

Repetitive studies of intra-arterial pressures after resection for coarctation of the aorta in man. BOWEN E. TAYLOR (by invitation), O. THERON CLAGETT (by invitation), HOWARD B. BURCHELL and EARL H. WOOD. *Section on Physiology, Mayo Foundation, and the Division of Surgery, Mayo Clinic, Rochester, Minn.* Continuous and simultaneous intraradial and intrafemoral arterial pressures and electrocardiographic data obtained from 17 patients before and after resection for coarctation of the aorta have indicated that, postoperatively, the cardiovascular dynamics were altered toward, but did not attain, normal values. Additional studies have been carried out to determine whether or not additional changes occur over longer periods after operation. In 10 patients studied preoperatively, radial and femoral arterial pressures averaged 199 systolic and 101 diastolic and 117 systolic and 84 diastolic, respectively. During the studies conducted an average of 3 weeks after operation, radial and femoral arterial pressures averaged 158 systolic and 77 diastolic, and 128 systolic and 74 diastolic, respectively. When these patients were again studied an average of 7 (3 to 11) months after operation, the radial arterial pressure had decreased on the average from the first postoperative study 16 ± 5 mm. Hg systolic and 9 ± 3 mm. Hg diastolic, although the average change in femoral systolic pressures was not significant. The average femoral diastolic pressure decreased 5.2 ± 2.5 mm. Hg. Significant improvement in the femoral/radial systolic pressure ratio and the femoral/radial pulse pressure ratio probably indicate that the decrease in cephalic blood pressure was related to a continued improvement in the blood flow through the descending thoracic aorta. Evidently, additional changes in the cardiovascular dynamics toward normal may continue over a period of months after resection for coarctation of the aorta.

Relationships between the blood pressure increases in exercise, carbon dioxide inhalation and the cold pressor test. HENRY LONGSTREET TAYLOR, AUSTIN HENSCHEL, JOSEF BROZEK and ANCEL KEYS. *Laby. of Physiological Hygiene, Univ. of Minnesota, Minneapolis, Minn.* The blood pressure responses to 3 standardized situations which increase blood pressure were studied in 379 normotensive healthy males. There were 159 subjects 18 to 26 years old and 220 men 45 to 54 years old. The tests were rigidly standardized as to time of events, relation to meals and psychological atmosphere. Blood pressures were recorded during a preliminary period with the subject supine. The hand was then immersed in ice water for one minute. Five minutes later, the subject breathed 6% carbon dioxide for 5 minutes. After a 5-minute recovery period, the subject walked on a motor driven treadmill at 3 miles/hour and 5% grade. Blood pressures were observed at frequent intervals during each test by the auscultatory method.

The greatest increase above the control level in the systolic blood pressure in each test was used as an index of the response. The product moment correlations for the following pairs were all less than 0.2 in both age groups: work systolic blood pressure increment and the cold test increment, work systolic blood pressure increment and the CO_2 test increment and cold test systolic blood pressure increment and the CO_2 test increment. There was no relationship between the resting systolic blood pressure and the systolic blood pressure increment in work or in the CO_2 test but the correlation between the resting systolic blood pressure and the systolic blood pressure increment in the cold test were 0.52 in the younger and 0.44 in the older age group.

Anti-diuretic substance in the urine after fainting. N. B. G. TAYLOR (by invitation) and R. L. NOBLE. *Dept. of Medical Research, Collip Medical Research Laby., Univ. of Western Ontario, London, Canada.* The present study was made on 14 normal young adult human male subjects, 7 of whom fainted and 7 of whom did not faint. They were bled by venesection of amounts from 270 to 1250 cc. There is no correlation between amount of blood loss and fainting. Urine specimens were obtained, by voiding, before and after fainting. These were extracted by the method of Noble *et al.* (1939) and tested for anti-diuretic activity by the rat method of Burn (1937). It has been established that the urine secreted after a faint contains an anti-diuretic substance in readily detectable amounts. No anti-diuretic activity is found in urine from *1*) normal subjects, engaged in routine duties about the laboratory (24-hour specimens); *2*) experimental subjects before venesection; *3*) experimental subjects after venesection, if they did not faint. Urine has also been obtained from 2 subjects who fainted spontaneously, i.e. psychic, not due to venesection, both these cases show anti-diuretic activity in the urine after the faint and not at other times. Evidence will be presented that the anti-diuretic substance found is the secretion of the posterior lobe of the pituitary gland.

Origin of renal vasoconstriction in tourniquet shock. R. D. TAYLOR and IRVINE H. PAGE. *Research Division and Bunts Institute of the Cleveland Clinic Foundation, Cleveland, Ohio.* We have previously reported on the renal vascular effects of application of tourniquets to the hind legs of dogs and concluded that the renal vasoconstriction which occurs is predominantly of humoral origin. The demonstrations of Trueta, Barclay *et al.*, impelled us to re-examine this conclusion. Observations were made in dogs in which afferent stimuli from the hind legs were blocked by section of the spinal cord at L. 1. The data thus made available for comparison consist of observations on renal circulation after application of leg tourniquets in *1*) normal dogs, *2*) in dogs with the kidneys denervated and *3*) in dogs with spinal cord section causing deafferentation. In contrast to normal dogs or dogs with denervated kidneys, dogs of the third group (deafferented) do not show significant changes in real vascular resistance or urine flow either during the time the tourniquets are in place or after their removal. Consequently, we conclude that the primary stimulus to renal vasoconstriction during the onset of tourniquet shock is neurogenic and arises at the site of tourniquet application. The presence of renal vasoconstriction in dogs with denervated kidneys suggests that the ultimate effector mechanism of this vasoconstriction is partly humoral.

Postural reflexes in deafferented limbs of the cat. R. D. TEASDALL (by invitation) and G. W. STAVRAKY. *Dept. of Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.* Sensitization of spinal neurones to chemical stimulating agents and to impulses reaching them via pyramidal tracts was demonstrated by Draks and Stavraky (*J. Neurophysiol.* 229: 11, 1948) and by Teasdall and Stavraky (*Federation Proc.* 7: 123, 1948). The present study of postural reflexes carried out by means of moving picture recordings showed that these reflexes though present in intact cats are not as distinct as in decerebrate animals being modified by voluntary movements and by a tendency of the forelimbs to stay in partial flexion. Within 1–3 weeks after deafferentation of one limb by means of posterior root section, postural reflexes, as noted by Bremer (*Annales de Physiologie* 4: 750, 1928), become very active and brisk. The responses are not only of the extensor type but also active flexion of limbs becomes prominent. Furthermore, in the supine position with labial cleft at 45° above the horizontal plane ('manimal attitudinal extensor position' of Magnus, *Lancet* 211: 531 & 585, 1926) the deafferented forelimb assumes a flexed position while the intact paw is extended at the radio-carpal joint. Ventroflexion of the head causes extension of the deafferented forelimb but usual flexion of the intact limb. Similarly, placement of the animal in the prone ('minimal') position increases the extensor tone of the deafferented forelimb. It is felt that these are modified positional and attitudinal reflexes brought about by impulses from the labyrinth and neck proprioceptors acting on spinal neurones sensitized by deafferentation. A differential study of these reflexes is in progress.

Metabolism of rat testis *in vitro*. JAY TEPPERMAN and HELEN M. TEPPERMAN (by invitation). *Dept. of Pharmacology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* In confirmation of the report of Schuler (*Helvet. Chim. Acta.* 27: 1796, 1944) it has been found that immature rat testis has a slightly higher Q_{O_2} without added substrate

than it does in the presence of glucose, whereas the mature gland exhibits a markedly diminished Q_{O_2} in the absence of glucose. These data may signify that a metabolic difference exists between the tubular and endocrine portions of the gland, since the adult organ contains a much larger proportion of functioning tubular tissue than does the immature one. To test this hypothesis, 11 adult rats (mean weight 287 gm.) were rendered unilaterally cryptorchid. Between 23 and 28 days after transplantation of the right testis, when histological examination showed marked regression of the tubular system, Q_{O_2} and representative dry weight determinations were made on the transplanted glands (mean weight, 155 mg/100 gm. of rat). The scrotal glands (mean weight, 495 mg/100 gm. of rat) from the same rats were used as controls. The results were as follows:

	Q_{O_2}	
	No substrate	200 mg. p.c. glucose
Transplanted (11 rats, 11 vessels)	6.24 S.E. ± 0.14	5.36 S.E. ± 0.20
Control (5 rats, 13 vessels)	3.95 S.E. ± 0.12	6.78 S.E. ± 0.12

It is suggested that the tubular and interstitial components of adult testis may be differentiated by the metabolism of the surviving tissue as described herein.

Reorganization of sensory function in amputation stumps: two-point discrimination. HANS-LUKAS TEUBER (by invitation), HOWARD P. KRIEGER (by invitation) and MORRIS B. BENDER. *Psychophysiological Laboratory, Dept. of Neurology, New York Univ. College of Medicine, New York City.* Thresholds of tactile discrimination of two points, simultaneously applied, were obtained in 36 men with unilateral amputations above the knee. All measurements were made with a touch compass applied to the skin of the stump and to homologous areas of the sound limb. Longitudinal thresholds were obtained by placing the compass along the proximo-distal axis of the limb; transverse thresholds by placing the points at right angles to this axis. The method of constant stimuli was used. The threshold was defined as that distance, between the two points of the compass, at which 50% of the responses were correct. Each threshold was determined by 140 trials. The thresholds for the stump were consistently below those obtained for homologous areas of the sound limb in 34 of the 36 subjects. The average transverse thresholds of the stump for all subjects were 24 mm. compared to 36 mm. on the sound limb. The average longitudinal thresholds were 29 mm. on the stump and 42 mm. on the sound limb. These differences were significant at the 1% level of confidence. Similar, though smaller, differences were found between the stump and sound limb on testing proximal parts of the stump, the differences decreasing with a definite gradient as the hip was approached. There were no indications that superiority in two-point discrimination of the stump was due to the stretching or folding of the skin or concomitant distortion of the peripheral patterns of its innervation. The results are considered as presumptive evidence for central readjustments which impart to the stump some of the functional characteristics of the amputated limb.

Ascorbic acid and acclimatization of animals to cold environment. M. THÉRIEN (by invitation) and L. P. DUGAL. *Dépt. d'Acclimatation, Institut de Biologie Humaine, Univ. Laval, Québec, Canada.* It has been shown in our previous studies, that the white rats, which are able to synthesize ascorbic acid, do react to a long exposure to cold by an important increase in the ascorbic acid content of their tissues if adaptation to the new surrounding takes place; if not, the contrary occurs, namely a significant decrease in the content of that vitamin. Following that observation it was found that guinea-pigs, which are unable to synthesize ascorbic acid, need more and more of that vitamin as the temperature of the surrounding is being lowered. Among interesting topics that have been observed, it may be said that: *a*) there is a retention of ascorbic acid in the cold, as indicated by analysis of the tissues and of the urine; *b*) a direct relation between ascorbic acid content of the adrenals and adaptability to low temperature; *c*) a preventive effect of ascorbic acid towards the normal hypertrophy of the adrenals in the cold.

Effect of vitamin deficiency, thyroid and adrenal hormones on oxidation enzymes of rat tissues. SAMUEL R. TIPTON and FRANCES M. COLVIN (by invitation). *Dept. of Zoology and Entomology, Univ. of Tennessee, Knoxville, Tenn.* We have extended our earlier work on the relationship of vitamins, hormones and respiratory enzymes (succinic dehydrogenase and cytochrome oxidase) to include adenosinetriphosphatase and d-amino acid oxidase of rat liver and kidney. Albino rats maintained on casein-sucrose-vegetable oil plus the necessary supplement of commercial vitamin B factors have somewhat lower liver and kidney phosphatase and oxidase activities than do rats maintained on Rockland Rat pellets. Administration of desiccated thyroid or thyroxine for 10 to 20-day periods results in an increase in the activity of both enzymes. After adrenalectomy there is a somewhat larger decrease in amino acid oxidase. Treatment with lipo-adrenal extract prevented the decrease in oxidase with a possibly significant rise in adenosinetriphosphatase. In vitamin B deficiency there is a small increase in liver phosphatase

while that of kidney is not significantly altered. There is a decrease in the activity of amino acid oxidase in both tissues. When the B-deficient animals are treated with thyroid powder there is a marked rise in liver phosphatase, the rise being considerably greater than that in hyperthyroidism in rats on stock diet. Kidney phosphatase does not show this B-deficiency effect. The stimulation of oxidase activity by the thyroid powder is depressed by B deficiency.

Syringe oxygen cathode for measurement of oxygen tension in solution and in respiratory gases. JULIAN M. TOBIAS. *Dept. of Physiology and Toxicity Laby., Univ. of Chicago, Chicago, Ill.* If the ends of a syringe plunger be removed, one has a hollow tube which fits tightly into the syringe barrel. It is then a simple matter to insert an insulated platinum wire and a pencil-type calomel cell so that their tips just protrude from the end of the opened plunger. The plunger is then filled with paraffin or a cement. With leads from the other ends of the electrodes the platinum wire can be made a cathode 0.8 v. negative to the calomel anode. If a solution be aspirated into the syringe, using the electrode fitted plunger as the piston, the current which flows can be shown to be a linear function of the oxygen tension of the solution. Precision has been studied using Ringer's solution. In a 0.5 cc. volume one can measure a change of 0.019 cmm. of O_2 with a standard error of 0.0001 cmm. With the volume reduced to 0.05–0.02 cc., using a 1-cc. tuberculin syringe, sensitivity is extended to 0.0008 cmm. of O_2 with a standard error of 1.7–2.3%. The instrument can also be used for measuring pO_2 in gas mixtures by taking in a small amount of Ringer and some of the gas. After equilibration, the pO_2 of the Ringer is measured. The instrument is not as precise as the Haldane analyzer, and at pO_2 values from 185–75 mm. Hg it may differ from the Haldane by 3.2 mm. Hg, on the average. An analysis takes a few minutes. The instrument has not yet been successfully used with blood.

Coagulation decelerating action of hemophilic plasma on normal plasma. L. M. TOCANTINS and R. T. CARROLL (by invitation). *Division of Hematology, Dept. of Medicine, Jefferson Medical College, Philadelphia, Penna.* A coagulation decelerating action of hemophilic plasma on normal plasma can be demonstrated if the bloods are collected swiftly, over suitable surfaces, centrifuged at high speed at 5°C. and the tests carried out in collodion or silicone coated tubes. This property of hemophilic plasma varies among hemophilic individuals and seems directly correlated with the severity of the defect and the degree of anticephalin activity of the plasma. Prolonged centrifugation in the cold accentuates the clot-decelerating power and anticephalin activity of the upper layers of the plasma, while it is being reduced in the lower layers. Dilution, aging and exposure to glass impair and eventually destroy clot decelerating and anticephalin activity. Normal plasma to which a lipid antithromboplastin (*Proc. Soc. Exptl. Biol. & Med.* 68: 110, 1948) has been added, acquires clot decelerating action on intact normal plasma, and behaves on dilution, and incubation with cephalin, like hemophilic plasma.

Effects of anticonvulsants upon properties of frog sciatic nerve. J. E. P. TOMAN, M. D. GREENHALGH (by invitation), J. S. CARLSON (by invitation) and G. H. BJORKMAN (by invitation). *Depts. of Physiology and Pharmacology, Univ. of Utah, College of Medicine, Salt Lake City, Utah.* Frog sciatic nerves were treated for 5 or more hours with solutions of various hydantoins, acetylureas, barbiturates, and oxazolidine-2,4-diones in Ringer's solution at *p*H 7.3 and room temperature. Those compounds having a 5-phenyl group and no sub stitution in the 3-N position were effective in concentrations of 1 mM or less in preventing the following normal phenomena: prolonged lowering of threshold and production of 'ultrasupernormality' and 'rebound spikes' following supramaximal stimulation; lowering of threshold and appearance of repetitive discharges after immersion in neutral isotonic phosphate solution. These phenomena are similar in some respects to the convulsive behavior of pyramidal neurones described by Adrian and Moruzzi (*J. Physiol.* 97: 153, 1939). Diphenylhydantoin, the most effective compound studied (ED_{50} = 0.04 mM), had little effect on other aspects of excitability and responsiveness. In occasional experiments there was a moderate increase in threshold and an abolition of the positive overshoot following a conducted spike. Diphenylhydantoin had no consistent effect upon membrane time and space constants, membrane potential, action potential amplitude, conduction velocity and recovery following submaximal stimulation. Although diphenylhydantoin did not alter the depolarizing and conduction blocking effects of KCl, it prevented the increased exchange of radioactive K and N_a ions in nerves pretreated with phosphate solution. The results indicate that some anticonvulsants may prevent abnormally induced hyperexcitability and high frequency discharges without impairing the normal function of neurones.

Effect of amino acids on neuro-muscular function. CLARA TORDA and HAROLD G. WOLFF. *New York Hospital and the Depts. of Medicine and Psychiatry, Cornell Univ. Medical College, New York City.* Since infusion of amino acids in patients with myasthenia gravis induce a temporary improvement manifesting itself in an increased performance of work, a decreased daily requirement of neostigmine, and, instead of the decline of the amplitude of action potential on indirect stimula-

tion characteristic for this disease, a maintenance of the amplitude of the action potential during indirect stimulation. To ascertain whether this beneficial effect was due to supplying an essential amino acid to muscle and nerve single amino acid deficiencies were established in mice to ascertain whether the deficient animal shows a myasthenia gravis-like impaired neuro-muscular function. Single amino acid deficiencies were established by administering crystalline amino acids, vitamins, fat, and carbohydrate as suggested in diets based on the work of Rose. Well fed mice, mice starved for 3 days, and mice kept on low rations of mixed food for 6 weeks served as controls. Mice starved for 3 days did not show an impaired neuro-muscular function as measured by electromyography. Mice kept on low food rations for 6 weeks showed some impairment of the neuro-muscular function. Mice on the diet containing the 10 essential amino acids showed a similar neuro-muscular function as mice on chronic starvation. Mice fed with diets containing 9 essential amino acids did not show an impaired neuro-muscular function after 2 weeks, showed some impairment after 4 weeks if the diet was lacking either leucine, or phenilalanine, lysine, histidine, threonine, methionine, and showed a marked impairment of neuro-muscular function after 6–8 weeks. Glutamic acid deficiency did not induce marked impairment of the neuro-muscular function.

Effect on the action potential of nerve and muscle of 2-methyl naphthoquinone. CLARA TORDA and HAROLD G. WOLFF. *New York Hospital and the Depts. of Medicine and Psychiatry, Cornell Univ. Medical College, New York City.* The effect of 2-methyl naphthoquinone on the function of nerve and muscle *in vitro* and *in vivo* was investigated to ascertain whether or not this potent inhibitor of choline acetylase impairs the function of nerve and muscle. In the presence of the naphthoquinone in concentrations of 1×10^{-5}M. the conduct on time of isolated nerve preparation increased and the area and amplitude of action potential decreased; the conduction time of nerve-muscle preparation (*in vitro* and *in vivo*) increased and the area and amplitude of action potential decreased. The muscle function on direct stimulation was not significantly impaired. Since naphthoquinone, in the concentrations used, is known to inhibit only choline acetylase, the dysfunction observed suggests that acetylcholine is necessary to maintain an optimal function of nerve, myoneural junction and the excitatory mechanism of muscle.

Gastric distention as a factor in the satiation of thirst. E. J. TOWBIN (introduced by E. F. ADOLPH). *Physiology Dept., Univ. of Rochester, Rochester, N. Y.* Since dogs stop drinking before any appreciable amount of water enters their blood, this study was designed to determine some of the factors in the satiation of thirst. Thirsty dogs with esophageal fistulae were allowed to sham-drink at hourly intervals. In some experiments a stomach-balloon was inflated with water before the dogs drank. The volume sham-drunk was two to three times in excess of the real water deficit, and water introduced into the stomach reduced sham-drinking (corroborating Bellows, 1939). Water introduced into the stomach-balloon was equally effective in partially inhibiting sham-drinking. Supra-diaphragmatic vagotomy abolished this effect in 3 of 5 dogs. Inflation of the balloon increases the interval between sham-drinks when there is free access to water. In other experiments, intact dogs had free access to water. Each drink was automatically recorded. Following supra-diaphragmatic vagotomy (2 dogs) the mean drink-size increased; in one dog it almost doubled. Bilateral splanchnicotomy and sympathectomy (D3 through D12), in 2 dogs, was followed by a reduction in the mean drink-size. Subsequent to vagotomy the drink-size rose above control values. To date, attempts to pick up vagal afferent impulses stimulated by gastric distention have been inconclusive. It is believed that the filling of the stomach initiates afferent impulses which are partial cues in gauging the amount of water drunk. Either these impulses ascend the vagal trunk or the atonicity subsequent to vagotomy interferes with the initiation of 'distention' impulses.

Thrombin formation in presence of proteolytic enzymes and platelets. BURTON L. TRAVIS (introduced by JOHN H. FERGUSON). *Dept. of Physiology, Univ. of North Carolina, Chapel Hill, N. C.* The plasma protease (Loomis *et al.*, *Arch. Biochem.* 12: 1, 1947), said not to activate prothrombin, clot oxalated plasma or purified fibrinogen (Loomis and Seegers, *Science* 104: 461, 1946), has been found *1*) to accelerate the clotting of recalcified plasma and *2*) to speed up the rate of thrombin formation from purified prothrombin activated in the combined presence of calcium (optimal), 'accelerator globulin' (AcG), and washed platelet suspensions. Small amounts of crystalline pancreatic trypsin showed apparently analogous effects, but these, unlike the 'fibrinolysin' actions, were inhibited by crystalline trypsin-inhibitor from pancreas and by the 'antifibrinolysin' of Loomis. It is unlikely, therefore, that the fibrinolytic protease itself if responsible for these accelerator effects. There is no thrombin demonstrable in the 'fibrinolysin' and it shows no accelerator effect in the absence of platelets. It does not accelerate prothrombin activation by tissue thromboplastin in the presence or absence of AcG.

Metabolism of radioactive iodine in severe anoxic anoxia. L. VAN MIDDLESWORTH. *Dept. of Physiology, Univ. of Tennessee College of Medi-*

cine, Memphis, Tenn. Several authors have shown that 40–50% reduction in oxygen consumption occurs in rats exposed to severe anoxic anoxia. This observation suggested the present investigation regarding the effect of anoxia on the thyroid activity as indicated by the alteration of radioactive iodine metabolism in anoxic rats. Fifteen young rats were placed on a low iodine diet and after 8 weeks 8 of the animals were exposed to 268 mm. Hg barometric pressure (simulated 27,000 ft. altitude) for 12 hours. All 15 of the rats were then injected with 10 microcuries of I^{131} and the anoxic rats were returned to the low barometric pressure. After 24 additional hours all of the animals were killed. The total I^{131} was determined in the thyroid gland, urine and a sample of plasma. The plasma I^{131} was fractionated by trichloracetic acid into 'bound' and 'unbound' I^{131}. Significant findings include a pronounced reduction in 'protein bound' I^{131} in the plasma of the anoxic rats. The average values (expressed as percentage of plasma I^{131}) for the male animals were as follows: trichloracetic acid filtrate of plasma of control rats, 26%, and of anoxic rats, 91%; trichloracetic acid precipitate of the plasma of the control rats, 74%, and of the anoxic rats, 8%. In these experiments the I^{131} content of the thyroid glands and urine was not significantly altered by the hypoxia.

Trial of enterogastrone in Mann-Williamson dogs. FRANK E. VISSCHER and STANLEY C. LYSTER (by invitation). *Labys. of the Upjohn Company, Dept. of Pharmacology & Endocrinology, Kalamazoo, Mich.* Two groups of six Mann-Williamson dogs were prepared; one group was injected intramuscularly five times weekly with 100 mg. enterogastrone; the other group was uninjected. Animals were maintained on a diet of specially prepared food containing 25 parts ground pancreas, 25 parts ground liver, and with the remaining 50 parts consisting of commercial dog food, bread, cod liver oil and milk. One gram of ferrous sulfate was added per day to the diet of each dog. Four of 6 injected animals survived in good condition; one of two deceased animals died of a perforated ulcer; the average survival time of the living animals was 8 months and of the deceased animals, 9 months. Three of 6 uninjected animals survived in good condition; 2 of 3 deceased animals died of a perforated ulcer; the average survival time of the living animals was 8 months and of the deceased animals, 5 months. These data indicate only an equivocal benefit derived from enterogastrone which had been prepared by the usual process involving precipitations by tannic and picric acids. The survival of 3 of 6 untreated animals may indicate the importance of adequate nutrition in the prevention of peptic ulcer. Data obtained at autopsy on the length of the duodenal transplant are also presented.

Effects of peroxidase on the Rh-antibodies. PHILIP F. WAGLEY (by invitation), IRWIN W. SIZER, LOUIS K. DIAMOND (by invitation) and FRED H. ALLEN (by invitation). *Dept. of Biology, Massachusetts Inst. of Technology, and Blood Grouping Laby., Children's Medical Center, Boston, Mass.* Observations have been made of the serological, physical and biochemical effects of peroxidase plus H_2O_2 on human serum containing anti-Rh agglutinins of specificity anti-D and anti-C (anti-Rh_0 and anti-Rh^1) active in both saline and albumin. Peroxidase activity against antibodies was considered of interest because it has recently been shown to oxidize certain proteins (Sizer, *Fed. Proc.* 6, 202, 1947) and because of the presence of this enzyme in phagocytic cells and its apparent absence in lymphocytes. There was significant diminution in titer of anti-Rh agglutinins after exposure for 6 hours at 37°C. (0.1 ml. serum) with as little as 300 μg. of peroxidase (having a P.Z. activity of 167) in the presence of H_2O_2 (0.3%). Hydrogen peroxide alone in concentrations as high as 0.3% had no observable effect. Manometric studies during the reaction revealed oxidation of serum proteins induced by the peroxidase-H_2O_2 system. Ultra-violet absorption spectra obtained before and after such inactivation of the antibodies by peroxidase showed changes in the absorption range of proteins. This suggests an oxidation by peroxidase of such constituent amino acids in protein as phenylalanine, tyrosine and tryptophane. These apparent peroxidase effects were not accounted for by any catalase present in the serum. Dialysis of serum against 0.85% saline at 6°C. for 72 hours prior to incubation with peroxidase did not prevent the effects of the latter on the antibodies. Purified anti-Rh globulin showed comparable changes induced by peroxidase when studied by serological, ultra-violet and manometric techniques. However, these peroxidase effects are not considered specific because anti-A and anti-B activities of human serum were also decreased by incubation with peroxidase in the presence of H_2O_2. Furthermore, serum containing no Rh-antibody during exposure to peroxidase-H_2O_2 showed manometric evidence of oxidation and alterations in ultra-violet absorption spectra of certain serum proteins.

Excitability changes of single visual receptor cells following flashes of light of intensity near threshold. I. H. WAGMAN, H. K. HARTLINE and LORUS J. MILNE (by invitation). *Dept. of Physiology, Jefferson Medical College, and Johnson Research Foundation, Univ. of Pennsylvania, Philadelphia, Penna.* The discharge of nerve impulses in single optic nerve fibers in the eye of *Limulus* has been studied in response to short flashes of light at intensities near threshold. At constant intensity a flash may or may not elicit a response; the responses were found to occur at random if the

flashes were 5 seconds or more apart. With a 2-second interval, the occurrence of a response created a bias against a response to the second flash. In a series of paired flashes of equal intensity separated by 2 seconds, with 10 seconds or more between successive pairs, responses occurred much less frequently to the second flash of the pair than to the first. This is true even of flashes following those flashes to which there had been no response, although the depression of sensitivity was less than when the first flash elicited a response. By varying the interval between flashes in the pairs, and by holding the intensity of the first flash constant while varying that of the second, it was found that within an interval up to about 1 second the effect of a subliminal flash summed with that of a flash following it. This was then followed by a condition of decreased sensitivity, the sensitivity reaching a minimum at 2 seconds (.1 to .2 log units below normal) and returned to normal by 5 seconds. Following a flash that had elicited a response, there was loss of sensitivity which disappeared in about 5 seconds.

Renin concentration of the kidney in experimental renal hypertension. G. E. WAKERLIN, JOHN MARSHALL (by invitation), R. O. BURNS (by invitation) and BESS G. OSGOOD (by invitation). *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago, Ill.* During the past several years in our study of experimental renal hypertension we have determined the individual renin concentrations of 500 dog kidneys. The renin concentration of the normal dog kidney is relatively constant at approximately 2.0 dog (Goldblatt) units per gm. of fresh tissue. Renin disappears from the contralateral kidney 20 days after unilateral renal artery constriction, remains absent for at least 100 days, and returns in normal concentration 10 days following homolateral renal artery constriction. Significant atrophy of the constricted kidney, injection of certain hog renal extracts containing renin, and extreme morbidity from distemper and/or pneumonia favor the return or presence of renin in the contralateral kidney. Unilateral nephrectomy appears not to change the renin concentration of the remaining kidney. The renin concentration of the kidney is probably normal 1–3 months following renal artery constriction and is increased by injections of purified hog renin. Prolonged renal artery constriction (5–78 months) tends to decrease the renin concentration of the kidney and significant atrophy may enhance this tendency, whereas distemper and/or pneumonia favor an increased renin concentration. The renin concentration of the constricted kidney of experimental malignant hypertension varies from subnormal to supernormal, is not changed by injections of crude hog renal extracts containing renin but is increased by injections of purified hog renin. There was no correlation between the renal renin concentration and the level of hypertension, chronic or malignant. Because of their limitations, these findings neither directly support nor oppose the renin-hypertension hypothesis of the pathogenesis of experimental renal hypertension.

Treatment and prophylaxis of experimental renal hypertension with renal extracts. G. E. WAKERLIN, J. E. BOURQUE (by invitation), JOHN MARSHALL (by invitation), R. O. BURNS (by invitation), BESS G. OSGOOD (by invitation) and LIONEL SCHOUR (by invitation). *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago, Ill.* We previously reported that crude hog renal extracts containing renin were highly effective in the treatment and prophylaxis of experimental renal hypertension in dogs. During the past four years we found various crude hog renal extracts and extract fractions to be variably and less effective. We now know that this difference is importantly due to the fact that our early extracts were prepared from hog renal cortex and our later less effective extracts were prepared from whole hog kidney. Thus all dogs of a group recently treated prophylactically with crude hog renal extract containing renin and prepared from cortex were protected against experimental renal hypertension, whereas no dogs treated with the same extract prepared from whole kidney were protected. Similarly, the same cortex extract was much more effective against experimental malignant hypertension than whole kidney extract. Purified hog renin (70 dog units/mg. N) was found to be effective in the treatment of renal hypertensive dogs. Purified hog renin was likewise effective in prophylaxis against hypertension but was definitely less effective than crude cortex extract in protecting against renal insufficiency and death following renal artery constriction to produce chronic hypertension. The results here reported are definitely favorable to antirenin as the mechanism of these therapeutic and prophylactic effects, but the question is still open and requires work now under way. The factor in renal medulla responsible for the inhibitory effect on the therapeutic and prophylactic potency of hog renal cortex and renin is also under investigation.

Enzymic reduction of the retinenes to the vitamins A. GEORGE WALD. *Biological Labys. of Harvard Univ., Cambridge, Mass.* Retinene$_1$, formed by the bleaching of the photosensitive pigment of rod vision rhodopsin, is subsequently converted to vitamin A_1 by a reaction in which reduced cozymase (DPN-H_2) acts as coenzyme. Two hydrogen atoms are transferred from DPN-H_2 to retinene$_1$, reducing its aldehyde group to the primary alcohol group of vitamin A_1 (Wald and Hubbard, *J. Gen. Physiol.* Jan. 20, 1949). The system which performs this reduction has now been isolated and fractionated into its components: the coenzyme DPN-

H_2; the substrate, synthetic retinene$_1$ (vitamin A_1 aldehyde) prepared by the oxidation of crystalline vitamin A_1; and the apoenzyme, retinene$_1$ reductase, extracted with dilute salt solutions from homogenized frog or cattle retinas. The apoenzyme is non-dialyzable, is precipitated by half saturation of its solutions with ammonium sulfate, and is destroyed by heating to 100°C. within 30 seconds. Its *pH* optimum lies at about 6.5. The rods of freshwater fishes contain a parallel system which reduces retinene$_2$, formed by bleaching porphyropsin, to vitamin A_2. This also can be put together from its isolated components in solution: retinene$_2$ reductase, extracted with dilute salt solutions from homogenized retinas; DPN-H_2; and as substrate synthetic retinene$_2$ prepared by the oxidation of vitamin A_2. These enzyme systems bring a member of the vitamin B complex, the anti-pellagra factor nicotinamide contained in cozymase, into the chemistry of rod vision. They introduce also a novel vitamin relation—a B vitamin participating directly in the regeneration of the vitamins A.

Pancreatic secretion in response to mechanical stimulation of the duodenum. J. M. WALDRON (by invitation), J. E. THOMAS and L. P. TKACZ (by invitation). *Dept. of Physiology, Jefferson Medical College, Philadelphia, Penna.* Three healthy adult dogs were provided with gastric and duodenal tubulated fistulas of the type described by Thomas (*Proc. Soc. Exp. Biol. & Med.* 46: 260, 1941). The duodenal fistula was placed opposite the main pancreatic duct and therefore the pancreatic duct could be easily cannulated with a fine glass cannula. The accessory pancreatic duct had been severed and doubly ligated. After complete recovery from the operation a total of 78 experiments were made on these 3 dogs to determine the effect of mechanical stimulation of the duodenum on pancreatic secretion. In the principle type of experiment fasting juice was collected for 1 hour, then the dog was fed 200 gm. of Purina Dog Checkers and pancreatic juice collected for 5 hours. In each dog experiments of this type were done with rubber tubes in the stomach and duodenum as the mechanical stimulus and without these tubes in place. Other experiments were performed on each dog to investigate the possibilities that increased HCl secretion or patent pylorus, or specific properties of the rubber tubing accounted for our results. These experiments conclusively demonstrate that mere mechanical stimulation of the duodenum changes the rate of pancreatic secretion in the fasting animal, usually increasing it. Furthermore the presence of mechanical stimuli in the duodenum alters the response of the pancreas to food.

Effect of temperature on the response of rat muscle to indirect repetitive stimulation. SHEPPARD M. WALKER. *Dept. of Physiology, Washington Univ. School of Medicine, St. Louis, Mo.* Isometric myograms were obtained from the triceps surae (in situ) of male rats under sodium barbital (300 mg/kg.) anaesthesia. Electrograms were recorded simultaneously from the gastrocnemius. The muscle temperature was reduced by refrigeration (10°C.) of the animals for 1 to 2 hours. At body temperature (36.5°C.) the peak tension induced by double shocks is 200 to 220% of peak twitch tension. The peak of added tension resulting from the second shock increases from 140 to 160% of peak twitch tension as the interval between shocks is increased beyond the refractory period to the early portion of the falling phase of the first response. Reduction of the muscle temperature to 26°C. induces a twitch with peak tension 180% and peak time 300% of normal. The peak tension produced by double shocks is only 133% of twitch tension in the cool muscle. The peak of tension added by a second response increases from 40 to 65% of twitch tension as the interval between shocks is increased beyond the refractory period to the peak time. The tension induced by tetanic stimulation at 125 shocks per sec. is slightly greater in warm muscle. Duration of the action potentials is prolonged in warm and in cool muscle by shocks repeated at short intervals. The results show that the cool muscle summates twitch tension less effectively than warm muscle both in double responses and in tetanic responses, although it shows a marked increase (180%) of twitch tension.

Effects of pancreatic duct ligation upon the action of secretin and pancreozymin in rabbits. C. C. WANG (by invitation), K. J. WANG (by invitation) and A. C. IVY. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago, Ill.* Rabbits with pancreatic ducts ligated for periods ranging from a few hours to two weeks were used. The functional state of the pancreas was assessed by studying the response to secretin and pancreozymin and correlations were made with the histological picture. Within 24 hours, the volume response to a standard secretin stimulation was clearly decreased and the enzyme content was lowered. Histopathologically, there were protoplasmic changes in the acinar cells and the ducts became dilated. On the fourth day, the effect of pancreozymin began to disappear and the secretin effect was greatly reduced. The enzyme content of the juice was very low. The corresponding tissue sections showed the progressive degeneration of the parenchyma and an infiltration of fibrous tissue. The response to secretin continued to fall progressively as the period of ligation was lengthened. Finally, on the 14th day, the parenchyma was mostly replaced by fibrous tissue and the secretin action was also gone. The scant juice obtained showed absence of amalyse.

Formation of protein or other aggregates by

reactions linked in series. DAVID F. WAUGH. *Dept. of Biology, Massachusetts Inst. of Technology, Cambridge, Mass.* The intermolecular bonds formed during collision of two single molecules may not be sufficiently strong to afford stabilization against thermal disruption. The association of three or more molecules, either by simultaneous collision or by single molecules colliding with previously associated groups, may be stabilized by the simultaneous formation of several low energy bonds. Although similar bonds may be formed throughout, the reaction course may be examined by assuming two reactions the first of which is termed an initiating (active center forming) reaction and the second the augmentation reaction (elongation reaction in fibril formation). The order of the first reaction indicates the number of molecules necessary to initiate a stable aggregate. The second is expected to be pseudo-first order with respect to unit aggregating surface, i.e., the rate at which 1 cm.^{2} of surface augments will vary with the concentration of free protein. The total augmenting area of each locus of aggregation may increase with size thus introducing a further kinetic complication. Such considerations give differential equations which have been solved for: first, an initiating reaction of order N followed by a pseudo-first order augmentation reaction assuming the aggregate rapidly approaches constant augmenting area; and second, an augmentation reaction alone in which predetermined numbers of active centers of initial size M_0 are introduced into solutions of protein and augment with increase in augmenting area. The general case of linked initiating and augmentation reactions with increase in augmenting area with size has been examined numerically.

Effect of increased renal venous pressure on renal function. RENÉ WÉGRIA and (by invitation) WILLIAM D. BLAKE, RICHARD P. KEATING and HENRY P. WARD. *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons and Presbyterian Hospital, New York City.* The effect of increased renal venous pressure on renal function was studied in anaesthetized dogs by means of clearance techniques. Renal function was measured separately and simultaneously in the two kidneys, but the pressure was raised in the left renal vein only, by means of a specially designed clamp. Moderate elevation of the left renal venous pressure from a normal value of around 100 mm. saline up to 350 mm. saline caused in that kidney a significant decrease in water and sodium excretion without any change in renal plasma flow, glomerular filtration rate, glucose Tm or diodrast Tm. The reduction in water and sodium excretion was due to an increase in the reabsorption rate of these substances by the renal tubule cells. This effect also occurred when the sodium load was low. Greater elevation of venous pressure (up to 550 mm. saline) decreased renal blood flow and filtration rate, but there were insufficient results to state whether filtration fraction was significantly altered. The mechanism for the increased reabsorption of sodium and water ascribed to the increase in venous pressure and the implications of these results on the pathogenesis of the edema of cardiac failure will be discussed.

Effects of the 'hyperglycemic factor' found in insulin preparations. H. F. WEISBERG (by invitation), R. CAREN (by invitation), B. HUDDLESTUN (by invitation) and R. LEVINE. *Dept. of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* This study was undertaken to evaluate certain aspects of the action of the 'hyperglycemic factor' (H.F.) present in commercial insulin preparations. *1)* Well-fed unanesthetized rabbits given 1–50 u/kg/hr. of insulin intravenously over a 6-hour period, demonstrated the presence of H. F. in all cases. However the degree of effect bore no consistent relation to dosage. *2)* Fasting (24 hrs.) abolished or greatly reduced the tendency to hyperglycemia. *3)* Subcutaneous injections of insulin into well-fed animals, in contrast to the intravenous route, resulted only in hypoglycemia. *4)* During the hyperglycemic phases of alloxan action in dogs there was no detectable increase in the H. F. of serum from the pancreatico-duodenal vein, as measured in vitro by the method of Sutherland and Cori (*J. Biol. Chem.* 172: 737, 1948). *5)* The part played by the adrenal medulla in determining the degree of hyperglycemia will be discussed. No physiological significance can, as yet, be attributed to this factor, on the basis of our findings or those in the literature.

Chemical structure and the physiological properties of acetylcholine. JOHN H. WELSH. *Biological Labys., Harvard Univ., Cambridge, Mass.* As a working hypothesis to explain the reactions between acetylcholine (Ach) and cellular constituents it has been suggested that Ach acts as a coenzyme. This requires postulating two classes of enzymes in order to account for the muscarinic and nicotinic actions of Ach. The nature and spatial arrangement of certain groups within the Ach molecule and the enzymes would determine, in part, which enzyme was activated. Pfeiffer (*Science*, 107 : 94, 1948) has correlated the spatial relationships of possible 'prosthetic' groups in Ach and certain parasympathomimetic drugs with the muscarinic activities of these compounds. This study is an attempt to determine the properties of the Ach molecule responsible for nicotinic action. The physiological actions of certain simple quaternary ammonium ions on invertebrate nerve and muscle have been observed and it has been found that at least three methyl groups are necessary for maximal nicotinic action. The substitution of 3

ethyl groups for the methyls attached to the nitrogen of Ach produces an effective Ach blocking agent which is stable to insect cholinesterase. In instances where choline esters, tetramethylammonium ions and anti-cholinesterases fail to produce excitation while triethylacetylcholine and tetraethylammonium ions excite, it is suggested that the level of active Ach within the tissue may be within the paralytic range and partial blocking of Ach may, in effect, reduce the level until it falls within the excitatory range.

A volumetric apparatus for studies of tissue metabolism. REIDAR WENNESLAND (introduced by J. FIELD). *Arctic Research Laby. Point Barrow, Alaska, and Dept. of Physiol., Stanford Univ., Calif.* A portable microrespirometer has been devised, which offers several advantages over the conventional Warburg type. These are: The volumetric principle, which allows a direct reading of the oxygen consumed, so that calibration of the respirometer vessels is unnecessary, less sensitivity to temperature changes, and a thermobarometer compensation for changes in barometric pressure and humidity. The measuring unit is compact, unbreakable and well suited for transport and field work. It is based on the same general principles as the respirometers worked out by Scholander. A V-shaped manometer and an oxygen chamber are drilled into a rectangular piece of plexiglass, (a material first introduced into microrespirometry by Scholander) and are connected to a respiration chamber of the Warburg vessel type. The second branch of the manometer is connected with a small thermobarometer bottle. The carbon dioxide produced is absorbed in alkali. The decrease in oxygen is indicated by the manometer. A stainless steel plunger is pushed into the oxygen chamber, until the manometer is back to the level it had at start and at previous readings. The position of the plunger can be read with a dial indicator or by a simplified micrometer screw device, and gives the oxygen consumed between the two readings, when the dimensions of the plunger are known. For a comparative study on tissue respiration by this and the Warburg method, see abstract by Peiss and Wennesland.

Blood pressure changes following explosive decompression to extreme altitudes. ATTIE YVONNE WERNER (by invitation) and SAMUEL GELFAN. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* By means of a strain gauge, continuous arterial blood pressure changes have been recorded in monkeys that were explosively decompressed from 20,000 to as high as 76,000 ft. and recompressed at free fall rate. The blood pressure may be reduced to less than one fifth of its predecompression level following such sudden reductions in pressure. This blood pressure reduction is a function of both the sudden decompression and anoxia. Following sudden decompression from sea level to 40,000 ft. simulated altitude in O_2, at which altitude the anoxia is insignificant, the slight blood pressure reduction is only of few seconds duration, after which both systolic and diastolic pressures are maintained at near control level while the animal remains at this altitude. The marked and sustained vasomotor change is observed only after sudden decompression to altitudes at which the animal is completely anoxic. We have confirmed the observation that the pulse pressure disappears after explosive decompression to altitudes above 60,000 ft. With relatively slow decompression to such altitudes, however, the pulse pressure is reduced but does not disappear. The systolic pressure may begin to recover during recompression when the animal is still at altitudes of complete anoxia, indicating a recovery from effects of sudden ambient pressure changes. The diastolic pressure recovery is slower and appears to be essentially a function of recovery from anoxia and very similar to records of recovery from 100% N_2 anoxia. This difference in recovery rates results in a temporary increase in pulse pressure above control level. Both systolic and diastolic pressures rise above predecompression levels towards the end of recompression but return to near control levels at end of recompression.

Renal dynamics and salt and water excretion in the dog following expansion of extracellular fluid. LAURENCE G. WESSON JR., W. PARKER ANSLOW JR., LAWRENCE G. RAISZ, ALFRED A. BOLOMEY and MICHAEL LADD (introduced by HOMER W. SMITH). *Dept. of Physiology, New York Univ. College of Medicine, New York City.* Extracellular fluid volume in dogs was increased 5 to 10% of body weight by slow or rapid infusion of modified Locke's solution and the increase sustained up to 9 hours by the intravenous replacement of NaCl, KCl and water as fast as these components were excreted. Filtration rate (C_F), renal plasma flow (C_{PAH}), filtration fraction (FF) and urine flow (V) showed a 3-phase response. During *Phase I*, C_{PAH}, C_F and V increased to peak values well above control values. At slow rates of infusion, C_F and C_{PAH} increased *pari passu*, FF remaining constant or decreasing. At rapid rates, increases in C_F were more rapid than increases in C_{PAH}. Increases in V paralleled those in C_F. During *Phase II*, C_{PAH} declined to control levels, and C_F to intermediate values so that FF was elevated. V declined approximately 50 per cent of the peak value in *I*. During *Phase III* C_{PAH} rose to values intermediate between peak and control values and C_F attained a level slightly below the peak value so that FF declined but did not fall below control values. V increased slightly in this phase. If the control values were low, the urine chloride concentration (U_{Cl}) rose to a peak dur ng *Phase I*,

then fell to a value slightly hypotonic to the blood shortly after the peak value of V was reached. If the control values were high, U_{Cl} decreased steadily to the hypotonic level. During *Phase III*, U_{Cl} rose until it attained a stable hypertonic level. In *Phase III*, renal dynamics, urine flow and urine concentration manifested a marked stability.

Renal tubular action of diuretics. II. Effects of mercurial diuresis on glucose reabsorption. R. E. WESTON, J. GROSSMAN (by invitation), I. S. EDELMAN (by invitation), D. J. W. ESCHER (by invitation), L. LEITER (by invitation), and L. HELLMAN (by invitation). *Medical Division, Montefiore Hospital, New York City.* The tubular site—proximal and/or distal—at which mercurial diuretics act has never been precisely localized. Recent investigations have demonstrated that in man salyrgan produces not only decreased tubular reabsorption of electrolytes and water, but also significant depression of TM_{PAH}, a renal function presumably limited to proximal tubular segments. Since it is well established that glucose reabsorption is limited to the proximal tubule, the effect of mercurial diuretics on maximal tubular capacity for glucose reabsorption (TM_G) was determined in human subjects without clinical renal disease. Preliminary experiments revealed that intravenous administration (2 cc.) of Mercuzanthin or Thiomerin (Disodium-N(γ-carboxy methyl mercapto mercuri-B-methoxy) propyl camphoramate) immediately after several ten minute control periods generally resulted in a 40–80% decrease in TM_G, which coincided with maximal electrolyte and water diuresis. As diuresis diminished, TM_G returned toward normal. In these earlier experiments, concentrated glucose-mannitol aqueous solutions were infused, and the combined mercurial-osmotic diuresis produced significant decreases in serum electrolytes and extracellular fluid. To obviate this, infusions were prepared in normal saline. Because prolonged (90–150 minute) administration of glucose in saline has been reported to produce 20–30% decreases in TM_G in occasional subjects, in a second series of experiments, control and post-mercurial glucose TM's were determined separately at 5–7 day intervals. In these shorter experiments, mercurials were administered prior to glucose infusion and produced similar depression of TM_G. It is concluded that in man mercurial diuretics depress a specific proximal tubular function.

Liberation of histamine by epinephrine. P. WHEELER (by invitation) and F. R. GOETZL. *Inst. of Medical Research, The Permanente Foundation, Oakland, Calif.* Recent reports indicate that in human beings an increase occurs in histamine content of blood plasma following intravenous administration of epinephrine. The observation was interpreted to indicate an ability of epinephrine to bring about liberation of histamine from tissues. The present *in vivo* and *in vitro* experiments were performed using rabbit's blood. Histamine was assayed according to the method of Barsoum and Gaddum as modified by Code. In 25 *in vivo* experiments epinephrine in doses varying from 0.05 to 1.0 mg/kg. was injected intravenously into anesthetized rabbits. Blood samples were obtained from the animals by heart puncture before and at 10 to 15 minute intervals after the injection for a period of 50 to 60 minutes. In blood samples thus obtained histamine was assayed and the leucocytes were counted. For the purpose of control similar experiments were performed in 10 animals which received intravenous injections of physiological saline solution instead of epinephrine. The results obtained from the in vivo experiments showed that intravenous injection of epinephrine causes a decrease in blood histamine and in the leucocyte count. In 37 *in vitro* experiments blood was obtained by heart puncture. Histamine was assayed in whole blood and in plasma after addition to the whole blood of equal volumes of epinephrine (0.001 and .01 mg/10 cc) and of saline solution, respectively. The results of the in vitro experiments showed that addition to whole blood of epinephrine is followed by an increase in plasma histamine, presumably due to liberation of histamine from leucocytes. The observations described are regarded to indicate that the decrease in blood histamine which, under the conditions of the experiments, followed intravenous injection of epinephrine into rabbits, was preceded by a shift of histamine from intracellular to extracellular compartments.

Development of seizure pattern by repetitive minimal cortical stimulation. J. A. WHIELDON (by invitation) and A. VAN HARREVELD. *Kerckhoff Labys. of the Biological Sciences, California Inst. of Technology, Pasadena, Calif.* Leão described a slowly spreading, transitory depression of the normal cortical activity which could be elicited by stimuli too weak to produce an afterdischarge. During this spreading depression he furthermore observed a cortical activity of a convulsive type. In the present experiments it was found that this convulsive activity is completely absent during the depression produced by the first stimulus after a long rest (1 hour). On repetition of the same stimulus at 5 to 10 minute intervals the convulsive activity developed and grew in intensity and duration. Also it was found that a stimulus of a strength and duration which initially was insufficient to produce an afterdischarge did so after repetition. Both afterdischarge and convulsive activity growing in intensity and duration with further repetition were in some experiments finally found to merge, producing an electroencephalogram typical of an electrically induced seizure. A rest of one hour was sufficient to restore the original situation

in which a stimulus produced spreading depression without either afterdischarge or convulsive activity. After a wave of spreading depression had passed over the cortex the cortical activity was abnormal (bursts or continuous runs of medium to high potentials, 100–500 micro volts; slow, 2-5/sec. waves) even if the stimulation produced neither afterdischarge nor convulsive activity.

The capacitance method for the study of cardiac activity. WILLIAM V. WHITEHORN and EDWARD R. PERL (by invitation). *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago, Ill.* Studies of capacitative changes accompanying the cardiac cycle in the human have continued. Substitution of A.C. for D.C. amplification in the apparatus has increased stability and gain and simplified operation. Techniques and conditions for record taking have been standardized and the relative importance of technical factors determined. Further analysis of records (cardiodielectrograms) in relation to simultaneously recorded electrical and mechanical cardiac events supports earlier conclusions that such records are directly related to cardiac volume changes and may provide an approximation of stroke volume. Comparisons of relative stroke volumes calculated from cardiodielectrograms of normal subjects under conditions of rest, exercise and performance of the Valsalva maneuver yield results in keeping with expected changes and provide evidence for the relative validity of the method. Absolute calibration of the instrument has been carried out by the introduction of spherical, saline filled balloons of known volume into the condenser field in positions approximating that of the heart. Results derived from such calibration curves cannot, however, be considered conclusively valid and comparison with simultaneous determinations by the direct Fick procedure are now in progress.

Action of dilute trypsin on muscle protoplasm (motion picture). FLOYD J. WIERCINSKI and BRIAN A. COOKSON (introduced by JOHN C. SCOTT). *Dept. of Physiology, Hahnemann Medical College, and Dept. of Zoology, Univ. of Pennsylvania, Philadelphia, Penna.* Recent studies have indicated that the colloidal behavior of muscle protoplasm is similar to the colloidal behavior of blood. The clotting of muscle protoplasm (for example by free Ca ion) produces shortening. Inasmuch as dilute trypsin solutions act like thrombin in causing blood clotting, it was thought to be of interest to determine the effect of dilute trypsin on muscle protoplasm. The trypsin preparation used contained 50% of a magnesium salt; the concentrations reported here are based on the trypsin content. The microinjection of a dilution of 1:1000 and 1:2000 in a calcium-free Ringer solution caused a dramatic clotting of the sarcoplasm but the sarcolemma was unaffected. The interior protoplasm contracted so sharply that it broke into pieces. A somewhat weaker effect is produced by the injection of a 1:10,000 solution. The myofibrils in the interior of the fiber were seen to break in several places along the longitudinal axis. The cortical region just under the sarcolemma appeared to be unaffected by this concentration of trypsin. A 1:100,000 dilution of trypsin upon injection into the muscle fiber caused very little effect but there was a slight contraction.

Testing the validity of ashing and digestion procedures by isotope dilution. WALTER S. WILDE. *Dept. of Physiology, Tulane University, New Orleans, La.* We analyze for phosphate in the phosphoprotein-nucleic acid residue of Schmidt and Thannhauser. The sulfuric-nitric acid digest (Fiske and Subbarow, *J. Biol. Chem.* 66: 377) of intestinal mucosa from the rabbit is analyzed colorimetrically. The procedure is tested by applying the expression, $P_d^*/P_d = P_{Mg}^*/P_{Mg}$. We call the known tracer mixed with the phosphate residue before digesting P_d^*. After digestion an aliquot is neutralized and precipitated as $MgNH_4PO_4$. This is washed thoroughly, redissolved in dilute acid, and analyzed for tracer and for phosphate (colorimetric). This gives the ratio P_{Mg}^*/P_{Mg}. We then calculate for P_d which is compared to a direct colorimetric analysis of another aliquot of the acid digest. Three runs from a common pool are tabulated:

P_d^*	P_{Mg}^*	P_{Mg}	CALC.	P_d FOUND
c/sec.	*c/sec.*	*mg.*	*mg.*	*mg.*
273	227	0.265	0.318	0.320
204	185	0.294	0.324	0.310
41	28	0.209	0.303	0.282

Although absolute loss occurs with the magnesia ppt. the tracer and non-tracer are lost proportionately so that their ratio remains unchanged and available for a valid calculation. The agreement between the value calculated and that found indicates there is no volatilization heat loss, that the presence of possible chemical interfering substances in the acid digest gives colorimetric results no different from those for the simpler solution of dissolved magnesia ppt., that pyrophosphates, if they do form, are later hydrolyzed.

Toxicity studies on antioxidants—butylated hydroxyanisole and hydroquinone. O. H. M. WILDER and H. R. KRAYBILL (introduced by A. J. CARLSON). *American Meat Inst. Foundation, Univ. of Chicago, Chicago, Ill.* Butylated hydroxyanisole (BHA) and hydroquinone (HQ) are both excellent antioxidants for use in animal fats. Toxicity studies have been made on BHA and on a mixture of BHA and HQ, using rats, to determine their LD-50s and to see if their long continued ingestion would have

any harmful effect on the animals. Six % of lard containing up to 2% of BHA was fed in a good basal ration for periods up to 21 months from weaning. No significant differences were observed in weight gains from weaning time between the controls and any of those getting the antioxidant, nor were there any differences in reproduction and growth of young. Another group of rats was started as soon after weaning as they would consume the ration and fed for 6 months at levels of BHA up to 2% of the ration. The only observed effect was a retarded weight gain at the higher levels. No effect was observed upon autopsy in any of the rats, and histological examination revealed no pathological conditions that could be attributed to the antioxidant at any level of feeding. A mixture of 80 parts BHA, 12 parts HQ, and 8 parts citric acid fed at levels of 0.3 and 2.0% of the ration has shown no effect during the growing period. The LD-50 of BHA dissolved in corn oil is 4.1 gm/kg. body weight in the rat. In a water emulsion, on a fasted rat, it is in excess of 5.0 gm/kg. body weight.

Pressure and flow of blood distal to a point of reduced arterial bore. ARNOLD H. WILLIAMS (introduced by HENRY A. SCHROEDER). *Dept. of Internal Medicine, and the Oscar Johnson Inst., Washington Univ. School of Medicine, and Barnes Hospital, St. Louis, Mo.* The decreases of pressure and flow of blood distal to a point of reduced arterial caliber are of considerable physiological importance. Heretofore studies of these hemodynamic changes, produced by external compression of an artery, have been complicated by obstruction of venous return or have been done without exclusion of collateral circulation. For this reason the changes of the pressure and flow of blood were studied in the brachial artery, distal to an adjustable clamp, in the isolated forelimb of five dogs. Blood was supplied from the animal but a perfusion reservoir was connected to the circuit to obtain pressure-flow curves. In one instance oxygen tension of the muscle was measured simultaneously. Short or prolonged arterial constrictions (up to 25 minutes) did not produce any consistent relationship between the reduction of pressure and the reduction of flow. Neither was this relationship found at the end of 15-second perfusions of the constricted artery. The calculated percentile decrease of flow was not always greater at lower perfusion pressures. Thus arterial constriction produced no consistent change of arteriolar resistance distal to the constriction.

Hepatic vital staining and dye excretion in CCl_4-treated mice. W. LANE WILLIAMS. *Dept. of Anatomy, Univ. of Minnesota, Minneapolis, Minn.* Hepatoxic centrilobular injury was produced by CCl_4 and concomitant hepatic reactions to the following were studied: *1*) two acid diazo dyes, trypan blue (Color Index 477) and chlorazol fast pink (C.I. 353); *2*) the triphenylmethane fluoran dye (also a xanthene fluoran), rose bengal (C.I. 779); and *3*) the sulfonated triphenylmethane dye, isamine blue (C.I. 710). Dyes were injected subcutaneously or intraperitoneally as 0.5 to 1% saline or aqueous solutions in amounts of 0.1 to 0.4 cc. The azo dyes were excreted in normal and CCl_4-treated mice by the kidney; Rose Bengal by the liver; isamine blue, by liver and kidney. The azo's and isamine blue were present in macrophages as segregated granules. Rose Bengal was not deposited in macrophages in such a fashion and did not appear in such cells in an observable quantity. The azo's and isamine blue clearly stained the cytoplasm of damaged cells and remained in such cells until lysis ensued. The former dyes were definitely aggregated into large particles during actual necrosis, but the latter did not clearly undergo such a striking alteration. In contrast to the above, Rose Bengal stained the cytoplasm of all hepatic parenchymal cells. At 4–6 hours after intraperitoneal injection normal cells were clear of this dye; while cells in the centrilobular area of damage retained the dye for 8–14 hours depending on the interval (24–48 hours) subsequent to treatment with the hepatoxic hydrocarbon.

Metabolism of the rat spinal cord functioning in isolation. R. D. TSCHIRGI (by invitation), R. W. GERARD, H. JENERICK (by invitation), L. L. BOYARSKY (by invitation), J. Z. HEARON (by invitation). *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* The perfused isolated spinal cord lends itself admirably to combined functional and metabolic studies. A technique has been developed for removing the rat thoracic cord supported on vertebral bodies, fixing in plaster, and perfusing (after Galli-Manini) with Tyrode plus glucose (100 mg. %) plus purified egg albumin and, sometimes, washed beef erythrocytes, at regulated temperature, pH, Co_2, C_{CO_2}, arterial, and pulse pressure. Flow averaged 1 cc/gm/min. Reflex activity, tested by stimulating a dorsal root and recording ventral root potentials, continued undiminished in experiments lasting over 3 hours. One minute asphyxia or 2 to 4 minutes perfusion without glucose abolished reflex response. Recovery was complete within 2 minutes on restoring oxygen or glucose, even after more than 30 minutes deprivation. Average glucose utilization, 7.5 mg/gram wet weight per hour (range 7.1 to 8.0), corresponds to a Q_{O_2} of 5000. Adrenalin increases the reflex response of a failing preparation. The areflexia of aglycemia can be used to test the ability of other substrates to support function. After 15 minutes inactivity without glucose (and later return with glucose) the following were ineffective, although several are easily oxidized: alcohol, acetate, lactate, β-hydroxybutyrate, fumarate, malate, succinate, adrenaline, dl alanine, dl lysine,

l tyrosine, dl aspartic, dl cysteine and l cystine. The following, in contrast, restored full function: pyruvate, oxaloacetate (partial restoration), isocitrate, α-ketoglutarate, glutamate and glutamine. Ammonia and glucose appeared in the perfusate with glutamate, glucose with α-ketoglutarate. Seemingly, successful metabolism of a substrate does not guarantee support of function.

Effect of abdominal ice packs on recovery from fatigue. W. W. TUTTLE, W. P. HAPP (by invitation) and M. WILSON (by invitation). *Dept. of Physiology, State Univ. of Iowa, Iowa City, Ia.* It has been reported that cold hip baths were employed in Germany during the last World War, in both industry and the air force, to ward off the onset of fatigue and to hasten recovery from it. It is the purpose of this investigation to gain further information relative to the beneficial effects of the local application of cold on recovery from fatigue. The problem was approached by comparing the maximum amount of work a subject would do on a bicycle ergometer in one minute of maximum effort before and after a 10-minute rest, both without and with an ice pack applied to the abdomen during the rest period. Eleven graduate men ranging in age from 25 to 35 years, and in weight from 130 to 200 pounds, served as subjects. A study of the data showed, that in every case, the drop-off in work following a rest period with an abdominal ice pack was significantly less than when the ice pack was omitted. The mean drop-off in work for the subjects when the abdominal ice pack was omitted from the rest period was 225 kg.M. and when ice was applied the mean drop-off was 69 kg.M. This difference is significant at the 1% level of confidence. It was also observed that for the most part, the application of abdominal ice packs between bouts of strenuous exercise alleviated the symptoms of exhaustion such as dizziness, nausea and muscular weakness.

Residual electrocardiograms. WILLIAM G. TURMAN (by invitation) and JANE SANDS ROBB. *Dept. of Pharmacology, Syracuse Univ. College of Medicine, Syracuse, N. Y.* In order to test the adequacy of our recording instruments for another study, various standard extremity, unipolar extremity and direct leads were recorded from turtles. Cardiac tissue was progressively cut away eliminating first the ventricle, then one and finally both atria. With the entire heart excised potentials from two pacemakers were still recorded. Inspection showed movement of the intrapericardial portions of right and left veins. These had to be resected far into the thorax before the galvanometer became quiescent. When but a few millimeters of one vein remained and pulsation of one spot, approximately 1 mm. sq., could be seen with a hand lens, potentials were still recorded from direct electrodes either paired to each other or with each paired to the central terminal which in turn was connected to 3 extremities. From this small area of pulsating vein, whose anatomical arrangement is relatively simple, Q-, R-, S-, and T-waves were registered. During this procedure there was a surprising maintenance of voltage suggesting considerable neutralization of current in the intact turtle. This study lends support to the view that the heart too has functional 'unit' areas which become activated, contract and produce each of the characteristic electrocardiographic waves. Although injury to some given area of the intact heart may alter the total electrocardiogram by altering summation, it seems unjustified to state that activation of some specified area is the cause of a given wave.

Effect of dinitrophenol on respiration of excised brains of rats at various stages of development. DAVID B. TYLER. *Dept. of Embryology, Carnegie Inst. of Washington, Baltimore, Md.* To test the hypothesis that the mechanisms involved in oxygen consumption may differ in brains of different ages, the effect of dinitrophenol, 10^{-5}M, was determined on the minced brain of rats, ranging in age from new born to adult, by means of Warburg respirometers. The oxygen uptake of the new born pallium (less than 24 hours old) is increased by an average of 28%. During the next 7 days there are indications that the sensitivity to the stimulating effect of this drug is diminished. The pallium of the 3-day-old rat shows only a 15% increase with the same concentration of dinitrophenol. Beginning about the 8th day there occurs a gradual increase in the response to dinitrophenol until the adult level is reached. Under the conditions of these experiments dinitrophenol (10^{-5}M) increases the oxygen uptake of the adult pallium by an average of 40%. No significant regional differences in response to dinitrophenol have been found. However, dinitrophenol enhances the depression of respiration produced by malonate or iodoacetate, depending upon the sensitivity of the region to the inhibitor. Thus the addition of dinitrophenol and iodoacetate to a suspension of minced adult medulla results in a lower oxygen uptake than when iodoacetate only is added, whereas a combination of dinitrophenol and malonate results in approximately the same oxygen consumption as occurs if malonate alone is added. In the case of the adult pallium a combination of dinitrophenol and malonate results in a significantly lower oxygen uptake than when malonate alone is used whereas the oxygen uptake of that region in media containing a combination of dinitrophenol and iodoacetate is approximately the same as when iodoacetate alone is used. The results with dinitrophenol support the hypothesis that the growth of the brain of the rat is accompanied by qualitative or quantitative differences in the systems responsible for its oxygen uptake.

Fibrinolysin activation by specific antigen, peptone and polysaccharides. GEORGES UNGAR (introduced by A. A. SCHILLER). *Rheumatic Fever Research Inst., Northwestern Univ., Chicago, Ill.* It is known that active proteolytic enzyme can appear when its inactive precursor present in serum is treated with chloroform or streptokinase. Activation has now been found to occur on addition of the antigen to serum from guinea-pigs previously sensitized to egg albumin. Activation was also observed in normal guinea-pig serum treated with peptone, agar, hyaluronic acid, chondroitinsulfuric acid, glycogen, pneumococcal polysaccharides and heparin. Higher concentrations of heparin however inhibit activation. The activating agent was added to serum which was then diluted with distilled water and adjusted to *p*H 5.4. The resulting precipitate, containing the enzyme, was centrifuged, redissolved and tested for proteolytic activity by a very sensitive fibrinogenolytic method and also by viscosimetry. Activation under the conditions mentioned above differs from the chloroform or streptokinase induced activation by the fact that it does not occur when the activating agent is added to the euglobulin precipitate or to serum previously heated to 56°. The findings reported here may have some bearing on the mechanism of anaphylactic and anaphylactoid reactions and on the functions of some high molecular carbohydrates.

Effects of explosive decompression on blood temperature in the right and left heart. EDWIN G. VAIL (by invitation) and FRED A. HITCHCOCK. *Dept. of Physiology, Ohio State Univ., Columbus, Ohio.* The development of the thermistor makes available an instrument for the precise measurement of small and rapidly occurring temperature changes. Experiments have already been reported in which thermistors were used to record temperature changes in the lungs. In the experiments reported in this communication, thermistors were inserted through the right jugular vein into the region of the right heart and through the right carotid artery into the region of the left heart. The exact location was determined by autopsy. Temperatures were recorded by means of a two-channel ink-writing oscillograph. The temperature in the right heart was found to be slightly higher than that in the left heart. There were slight fluctuations in the temperature of the right heart which could be correlated with respiratory movements and may possibly be due to the proximity of the superior vena cava to the respiratory passages. No temperature fluctuations were observed in the left heart. Following explosive decompression to 30 ± 5 mm. Hg there was an immediate drop in the temperature of the right heart and two or three seconds later, a drop in the temperature of the left heart. This decrease in temperature was greater in the left than in the right heart. The maximum drop in temperature occurred in about 20 seconds.

Studies on the effect of anoxic anoxia on sheep blood sugar and hemoglobin. EDWARD J. VAN LIERE, J. CLIFFORD STICKNEY and DAVID W. NORTHUP. *Dept. of Physiology, West Virginia Univ. School of Medicine, Morgantown, W. Va.* Sheep were chosen for these experiments because of their known low fasting blood sugar. Two castrate male Corriedale lambs (*no. 32:* 23.6 kg. and *no. 53:* 27.4 kg.) were studied in regard to blood sugar and hemoglobin in jugular vein samples drawn after a 24-hour fast under control conditions and before and after 30-minute exposures at weekly intervals in a decompression chamber to simulated altitudes. Blood sugar was determined colorimetrically on Somogyi-Shaffer-Hartmann filtrates and hemoglobin by the Sahli method. The average control blood sugar in the 2 sheep was 52 and 53 mg/100 ml. *Sheep 32* responded to a 30-minute stay in the decompression chamber at ground level with an average rise of 10 mg/100 ml. in blood sugar in 4 tests. *Sheep 53* in 3 tests showed no further rises after the first. The first exposure to 24,000 ft. produced rises in blood sugar of 40 and 22 mg/100 ml. in *sheep 32* and *53* respectively. Subsequent exposures to altitudes from 18,000–28,000 ft. produced no significant blood sugar rises in *sheep 32*. Similar exposures in *sheep 53* produced rises of 6–18 mg/100 ml. Blood hemoglobin was also followed before and after exposure to anoxia. *Sheep 32* consistently responded with an increase in hemoglobin concentration. *Sheep 53* responded at first with small elevations, but later with reductions in hemoglobin concentration.

Pharmacology of N, 1 dimethyl hexylamine (Oenethyl). J. H. WILLS, *Dept. of Pharmacology, Univ. of Tennessee, Memphis, Tenn.* In the dog anesthetised with pentobarbital sodium, N,1 dimethyl hexylamine (Oenethyl) has both depressor and pressor actions, the latter predominating. The pressor action appears to have a dose-effect curve of the general form $Y = A + BX$; it is blocked by ergonovine but not by ergotamine or ergotoxin. Oenethyl increases the tonus of both jejunum and uterus *in situ*. The drug produces premature ventricular contractions but fibrillation has not been seen. The rate and depth of respiration may be increased by Oenethyl.

Permeability of rabbit leucocytes to sodium and potassium. D. L. WILSON (by invitation) and J. F. MANERY. *Dept. of Biochemistry, Univ. of Toronto, Toronto, Canada.* Rabbit leucocytes were suspended in buffered solutions, the osmotic pressure of which was largely supplied either by sucrose or ammonium chloride. In the sucrose solution the chloride and potassium concentrations of the cells decreased 80% and 50% respectively. In the ammonium chloride solution the intracellular potassium decreased 90%, the intracellular sodium about 80%. The permeability to sodium was further studied by examining the penetration of Na^{24}

During the first 75 minutes after exposure to the isotope the specific activity (i.e. counts/min/m. Eq.) of the sodium inside the cell was 90% of that outside. In addition, data were obtained which show that the external concentration of glucose influences the intracellular sodium and potassium concentrations of leucocytes.

Inhibition of growth of mice on a low protein, choline deficient diet by coramine. J. WALTER WILSON and ELIZABETH H. LEDUC (introduced by PAUL F. FENTON). *Brown University, Providence, R. I.* Weanling mice were fed a low protein (4% casein) choline deficient diet, and the same diet with coramine. Without coramine, they grew slowly to nearly adult size and were still gaining slightly at 200 days. They had very fatty livers. With coramine, growth was greatly inhibited. With 1% coramine a gradual loss of weight occurred for 15 days. The mice were then fed 3 mg. of choline by stomach tube whenever they showed signs of decline. They were maintained 64 days with no gain of weight. There was no fat in the liver. With 0.5% coramine growth was completely inhibited, but the mice were maintained 84 days without supplementary choline. With 0.25% coramine a slight but varied growth occurred but it did not approach that on the basal diet. The livers contained considerable fat. The basal diet contains a minimum of labile methyl groups. Detoxication of coramine occurs by transmethylation. 0.25% coramine added to the diet leaves just enough labile methyl groups for very slight growth; 0.5%, just enough for maintenance without growth; 1% requires so many that not enough are left for maintenance and the animal declines. This can be corrected by choline. This effect of choline cannot be attributed to its lipotropic action, for on 1% coramine the liver is not fatty. The choline must supply methyl groups. On a low protein diet then, choline may supply methyl groups for the detoxication of excess coramine, thus permitting maintenance during prolonged inhibition of growth.

Effect of antihistaminic drugs upon trained rats. CHARLES A. WINTER and LARS FLATAKER (by invitation). *Merck Inst. for Therapeutic Research, Rahway, N. J.* Antihistaminic drugs affect central nervous system activity, as is evident from the side reactions observed in man, but this effect has not been much studied in animals. We have previously shown that antihistaminics potentiate the action of barbiturates. In the present experiments, fasted rats were trained to climb a vertical rope a distance of 172 cm. Antihistaminics and related drugs in doses of 5 to 25 mg/kg. intraperitoneally markedly affect the rate of climb. In many instances the animals fail completely. Drugs such as curare, which exhibit peripheral effects, also affect climbing time, but there is a qualitative difference in behavior of the animals. Partially curarized animals (150 micrograms/kg. of d-tubo-curarine) attempt to climb as do untreated rats, but they appear to be weak and easily become fatigued. Sufficient doses of antihistaminic drugs, on the other hand, make the animal become confused, and unable to decide whether to climb or not. It is therefore tentatively assumed that the results are a demonstration of central action of antihistaminic drugs. The central mechanism involved may not be the same one that was measured in experiments on barbiturate potentiation, because the compounds do not fall in the same order of graded activity in the two experiments. Two compounds having especially strong activity in the rope climbing test are Phenergan, N-β-dimethylamino-α-methylethyl)-phenothiazine, and Diparcol, N-(β-diethylaminoethyl)-phenothiazine. Both of these compounds have been reported to be useful in the treatment of Parkinsonism.

Effect of potassium deficiency upon gastrointestinal motility. HELEN A. WINTER (by invitation), HEBBEL E. HOFF and LESLIE DSO (by invitation). *Dept. of Physiology, McGill Univ., Montreal, Canada.* The motility of the gastrointestinal tract has been investigated in rats during potassium deficiency, and the therapeutic effects of potassium have been shown. Potassium deficiency was induced by reducing the intake of potassium or by injecting desoxycorticosterone. Synthetic diets were given ad libitum or by stomach tube. Observations on motility were made by X-ray and by inspection of the gut and were correlated with studies of potassium metabolism. In potassium deficient rats the tone and rhythmicity of the whole gut were reduced, particularly in the small bowel and stomach. Gas and fluid accumulated in the flaccid tract. Evacuation of barium was very slow. While normal animals evacuated barium from the stomach in about three hours and excreted more than one-half in five hours, deficient rats usually retained some barium in the stomach for a day and often excreted little barium in this time. In some the cecum reached gigantic proportions. In force-fed animals tremendous distention developed suddenly which proved fatal in all untreated rats. Therapy with potassium relieved the symptoms in surprisingly short time. In animals injected with potassium, rhythmic contractions were initiated in the small bowel within an hour and most of the barium was evacuated in less than a day. In advanced deficiency a change in the diet to one with a normal potassium concentration restored the motility of the gut to, or almost to, normal within a week. Later some animals showed slight regression in the improvement which may have been due to the diet or laboratory conditions. In most, recovery of normal motility could then be obtained on a diet of purina.

Treatment of peptic ulcer with desiccated defatted whole duodenum—Report of failure. C. W. WIRTS (by invitation) and M. H. F. FRIEDMAN.

Jefferson Medical College, Philadelphia, Penna. All subjects were patients with proved duodenal ulcer. Treatment periods ranged from 11 to 15 months and data are adjusted for a 12-month period. *Series A.* 34 patients received orally 12 to 15 gm. of dried defatted duodenum substance per day, plus ulcer diet. Average number of 'ulcer attacks' per year were 3.1 before treatment and 0.4 after treatment. X-ray evidence of ulcer healing was not obtained in 4 cases. *Series B.* 15 patients received 12 gm. enteric coated lactose tablets per day plus ulcer diet. Average number of 'ulcer attacks' per year were reduced from 3.2 to 0.1. All patients showed X-ray evidence of ulcer healing. *Series C.* 18 patients received ulcer diet alone. Average number of 'ulcer attacks' per year were reduced from 3.3 to 0.5. One patient gave no X-ray evidence of ulcer healing. It is concluded that, when given daily for a period of 11 to 15 months in doses approaching the maximum tolerated by the patient, dried defatted whole duodenum did not prove to be of benefit to the patient with peptic ulcer.

New evidence on the nature of cutaneous hyperalgesia. HAROLD G. WOLFF, H. GOODELL (by invitation) and JAMES D. HARDY. *New York Hospital and the Depts. of Medicine, Psychiatry and Physiology, Cornell Univ. Medical College, New York City.* Two important types of Hyperalgesia in the skin were identified as regards their special characteristics: *1*) That occurring at the site of skin injury characterized by lowered pain threshold, increased sensitivity to noxious and ordinarily non-noxious stimuli and termed for convenience primary hyperalgesia. *2*) Secondary hyperalgesia, occurring in areas of undamaged skin characterized by unchanged pain threshold but increased sensitivity to noxious stimulation. Development of secondary hyperalgesia has been observed following prolonged stimulation at or just below the pain threshold. Such hyperalgesia ends immediately on termination of stimulation. Temporary obliteration of secondary hyperalgesia can be accomplished by pin pricks within the zone of secondary hyperalgesia. Spatial summation of noxious impulses has been observed to be a characteristic of painful stimulation in the zone of secondary hyperalgesia. All of the above evidence further supports the view that secondary hyperalgesia is the result of a central excitatory state maintained by a flow of noxious impulses from the periphery.

Effect of altered metabolism on extreme hypothermia in dogs and albino rats. RICHARD C. WOLFF (by invitation) and K. E. PENROD. *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Twenty-nine rats fed 0.03% propylthiouracil in the diet for 24 days, 30 fed 0.12% thyroid extract for 9 days, and 28 controls were cooled by immersion to the shoulders in water of 7°C. Twenty rats receiving 0.03% propylthiouracil for 30 days, 16 receiving 0.50% thyroid extract for 15 days and 20 controls were cooled in a similar manner. The rats which were fed the propylthiouracil diet showed a metabolic depression of 15% while the group fed a 0.12% thyroid diet showed a stimulation of 14% and those receiving the 0.50% diet showed a stimulation of 41%. The first group of 87 rats was cooled to a rectal temperature 15.8-16.0°C. Sixty-nine per cent of the hypothyroid, 75% of the control and 63% of the hyperthyroid rats survived. The second group (56 rats) was cooled to a rectal temperature of 14.8–15.0°C. Forty-nine per cent of the hypothyroid, 50% of the control and 38% of the hyperthyroid rats survived. The rate of cooling to a rectal temperature of 20°C. in a bath of 4°C., the time of rewarming in water and air to 34°C. and the oxygen consumption were measured on 6 dogs, twice each. Two of the dogs succumbed in 'rewarming death'. The 4 surviving dogs were fed 4 mg/kg. thyroid extract for 13 days and again cooled. Only one dog showed an increased metabolism and all showed an increased rate of cooling. In 2 cases the rewarming time was lessened, in 2 increased. It is concluded that during immersion in very cold water the metabolic defenses of the animal are not capable of significant protection.

Rapid methods for enzymatic estimation of nucleoprotein intermediate metabolites by differential colorimetry. W. Q. WOLFSON (by invitation), C. COHN, R. LEVINE and K. KADOTA (by invitation). *Depts. of Biochemistry and of Metabolic and Endocrine Research, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.* Since the purine intermediates in nucleoprotein breakdown eventually give rise to urate when exposed to suitable enzymes, the methods here proposed consist of measuring the quantitative appearance of urate (colorimetrically) after incubation of the sample with known enzyme solutions. Enzyme purification need only be carried far enough to yield a liquid preparation of satisfactory specificity which gives no blank in the determination and may be stored frozen. The procedures now in use include: *1*) *True urate*, estimated from urate disappearance after treatment with beef kidney uricase. *2*) *Oxypurine* (*xanthine plus hypoxanthine*), estimated as urate appearing after treatment with xanthine oxidase from raw milk. *3*) *Purine nucleoside plus oxypurine*, estimated from urate appearing after treatment with xanthine oxidase and nucleosidase from human liver. The latter has adenosine desaminase activity, but has little guanase and no adenase activity. *4*) *Purine nucleoside*, calculated by subtracting #2 from #3. *5*) *Purine nucleoside, aminopurine and oxypurine*, estimated from urate appearing after treatment with xanthine oxidase, nucleosidase and crude adenase-

gunnase from Esch. coli. *6) Aminopurine*, estimated by subtracting #3 from #5. Apart from their simplicity, these procedures have the advantage of being relatively free from interference by side-reactions, such as the conversion of methylxanthines to methylurates during xanthine oxidase treatment. An example of the application of one of these methods to physiological problems is given elsewhere in these proceedings.

Metabolism of oxypurines in man: preliminary observations. WILLIAM Q. WOLFSON (by invitation), CLARENCE COHN and KINU KADOTA (by invitation). *Dept. of Biochemistry, Medical Research Inst., Michael Reese Hospital, Chicago, Ill.*

In man, the physiological course of intermediate purine metabolism prior to urate is largely unknown. The liver may be the chief site of urate production since it is believed to be the sole locus of xanthine oxidase, and because extraordinarily low blood urate levels have been reported in acute yellow atrophy. Oxypurines might be produced in the periphery and transported to the liver for conversion to urate, about $\frac{1}{15}$ being excreted by the kidney. If so, a significant increase in serum oxypurine should occur in hepatic, but not in renal, insufficiency. However, a large portion of the oxypurine might be formed as well as metabolized in the liver; and, in this event, oxypurine precursors rather than oxypurine should be retained in hepatic insufficiency. It has also been suggested that gouty hyperuricemia might be associated with a failure of inhibition normally exerted on hepatic xanthine oxidase. Serum and urine oxypurines have been determined by differential enzymatic colorimetry. In 14 normal adults, serum oxypurine averaged 0.30 mg. %. (S.D. $\pm$ 0.14 mg. %). Serum oxypurines were not elevated in pooled sera from patients with liver damage or renal insufficiency, but these data are not sufficiently consistent to permit definite conclusions as yet. The average serum oxypurine in repeated samples from five gout patients averaged 0.11 mg. %. This result is consistent with the suggestion that there may be a relative hyperactivity of hepatic xanthine oxidase in gout.

Oximetric measurement of circulation time and arterial saturation time during oxygen breathing in man. EARL H. WOOD, BOWEN E. TAYLOR (by invitation) and JULIAN KNUTSON (by invitation). *Section on Physiology, Mayo Foundation, Univ. of Minnesota, Rochester, Minn.* Arterial saturation was recorded continuously and simultaneously both at the ear and wrist (abstract J. Knutson et al) in 6 subjects as the gas mixture breathed was changed from air to 40% O_2, from 40 to 99.6% O_2, and from air to 99.6% O_2. After changing from air to 40% O_2 and air to 100% O_2, the initial increase in saturation (circulation time, Matthes) occurred at 6.2 (3 to 9) and 9.6 (6 to 12) seconds at the ear and wrist, and the maximal plateau value was attained at 47 (19 to 74) and 46 (19 to 71) seconds, respectively. Time required to increase from 97.9% to the plateau value minus 0.5 % (Fowler and Comroe), averaged 25 (11 to 58) seconds at both the ear and wrist, as compared to 19 (14 to 28) seconds (ear) and 25 (18 to 30) seconds (wrist) required to increase from 71 to 97 per cent after termination of breath-holding with helium (abstract J. Knutson et al). Changing from 40 to 100% O_2 produced an increase in arterial saturation both at the ear and wrist, averaging 0.40 (0.2 to 0.5) and 0.35 (0.1 to 0.7) %, respectively. The approximate arterial O_2 tension calculated from the physically dissolved oxygen (O_2 content—O_2 capacity) averaged 213 and 595 mm. Hg when 40 and 100% O_2, respectively, was breathed. The similarity in time and the magnitude of changes in arterial saturation recorded photo-electrically at the ear and directly on the arterial blood at the wrist indicate that an ear oximeter can be used successfully for determination of circulation and arterial saturation times in man.

Passive sensitizationof normal dogs by thoracic duct lymph from dogs sensitized to horse serum. PARKE H. WOODARD (introduced by K. E. JOCHIM). *Dept. of Physiology, Univ. of Kansas, Lawrence, Kan.* It is known that normal dogs may be passively sensitized to horse serum by transfusing into them blood from dogs previously sensitized to horse serum and that they will exhibit some of the symptoms of anaphylactic shock upon intravenous injection of horse serum. The symptoms most generally reported have been a fall in arterial blood pressure and changes in blood coagulation time. I have been able to confirm previous work from this laboratory that such passive sensitization is immediate and does not require lapse of time as has been reported by some workers. Not all sensitive animals are capable of passively sensitizing normal animals. In those which are capable it was thought worth while determining whether or not antibodies were distributed in other body fluids than blood. Accordingly dogs were sensitized to horse serum. Each animal was anesthetized with nembutal and the thoracic ducts cannulated and sufficient lymph collected for injection into a second anesthetized dog. In most cases blood was also drawn from the sensitized animal and transfused into a third anesthetized dog. Blood pressure records and blood clotting times were recorded on all animals before and after the administration of shock doses of horse serum. Eight control experiments were run in which the lymph and blood from normal dogs was similarly tested. These showed no evidence of shock. Of 11 experimental animals, 2 were not sensitive, 4 were sensitive but did not passively transfer immunity either through blood or lymph, 1 showed passive transfer through

blood but not lymph, 4 showed evidence of passive transfer by both blood and lymph.

Antagonism of adrenocorticotrophic hormone (ACTH) to desoxycortico-costerone acetate (DCA) on electroshock threshold and electrolytes. Dixon M. Woodbury (by invitation), Chi-Ping Cheng (by invitation) and George Sayers. *Depts. of Physiology and Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah.* By the 12th day after implantation of DCA (six 15 mg. pellets into each of 14 rats) the electroshock threshold had risen to +17%; 14 controls showed no significant change (+2%). Beginning on the 12th day, 7 of the DCA-implanted rats and 7 of the controls were injected twice daily for ten days with 2 mg. of ACTH. On the 22nd day the electroshock thresholds of the four groups were as follows: *I*) control, +3%; *II*) ACTH, +6%; *III*) DCA, +22%; *IV*) DCA +ACTH, +5%. When ACTH was withdrawn from group *IV* the threshold rose to +17% by the 32nd day. Readministration of ACTH resulted in a threshold reduction to +10% after 9 days; *III* had an average threshold of +24% at this time (41st day). DCA elevated plasma sodium and reduced plasma potassium and chloride. ACTH partially corrected the deranged electrolyte pattern of the DCA-treated rats. Whole extract of adrenal cortex restored to normal the elevated threshold of DCA-implanted rats. Since ACTH did not influence the elevated electroshock threshold of adrenalectomized rats implanted with DCA, the trophin acts via the adrenals. The results are interpreted to mean that DCA in intact animals, produces a relative deficiency of cortical steroids with an O on C-11 by inhibiting the release of ACTH from the pituitary.

Early components of contralaterally evoked cortical potentials in the intact unanesthetized rat. Lowell A. Woodbury (introduced by J. E. P. Toman). *Dept. of Physiology, Univ. of Utah College of Medicine, Salt Lake City, Utah.* As a preliminary to a study of the action of central excitant and depressant drugs, control observations were made on the initial components of the electrocorticogram evoked by stimulation of the contralateral hemisphere in ten unanesthetized intact rats. Epidural insulated screw electrodes were implanted under anesthesia one or two days prior to each experiment. Brief rectangular pulses or condenser discharges were delivered to the visual cortex of one hemisphere, while responses were taken from the symmetrical contralateral area. The characteristic initial response to submaximal stimuli consisted of two initially surface-positive spikes and a third surface-negative spike with latencies of 1.8–2.0, 3.4–3.6, and 4.0–4.2 msec. respectively. A voltage-response study of the large third spike showed a relatively homogeneous response population. Strength-duration curves for this response gave a limiting log-log slope of −0.7 and a chronaxie of 0.18 msec. The recovery curve following maximal responses exhibited relative refractoriness to 2.5 to 3.0 msec. followed by a period of greatly enhanced excitability with a peak at 4.0 to 6.0 msec. Since this phase was absent when conditioning responses were reduced below the amplitude of test spikes, it is attributed to a true supernormal period of previously responding elements rather than to indirect facilitation. This phase was followed by subnormal excitability with a minimum in the vicinity of 10–20 msec. and further minor oscillations at equal log-time intervals to at least 400 msec.

The pattern of localization in the motor cortex of the marmoset (Hapale jacchus). C. N. Woolsey, P. H. Settlage (by invitation), H. M. Suckle (by invitation) and W. G. Bingham (by invitation). *Depts. of Physiology, Anatomy and Surgery, Univ. of Wisconsin, Medical School, Madison, Wis.* We have examined in detail the motor area of this primitive new world monkey in order to compare the pattern of organization with the relatively simple plan found in the rat (see abstract by Settlage et al.). Certain comparisons are possible, but the main features of the primate arrangement have already been established. The relation of the motor pattern to that of the postcentral tactile area as defined by the evoked potential method (unpublished data, C. N. W.) will be considered.

Actions of dihydro-B-erythroidin and dtubocurarine on neuromuscular and synaptic transmission in frog and crayfish. Ernest B. Wright (introduced by W. O. Fenn). *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* 2 mg/kg. dihydro-B-erythroidin (eryth) and 4 mg/kg. dtubocurarine (dtubo) paralyze the frog in 20 and 30 minutes respectively, neuromuscular transmission being totally blocked. Neuromuscular transmission in the isolated sciatic-sartorius is blocked by immersion in 5×10^{-7} eryth and 5×10^{-6} dtubo with reduction of the endplate potential. Action potentials in the crural nerve and contractions of the cruralis muscle with blood supply tied off, elicited by stimulation of the foot skin, knee skin or sciatic nerve show that: *a*) sensory endings and nerve conduction are unaffected by both drugs; *b*) transmission of flexion reflex is unaffected or slightly enhanced by dtubo, greatly depressed or blocked by eryth. The crayfish is unaffected by 50 mg/kg. dtubo, but paralyzed in 2 minutes by 0.05 mg/kg. and in 10 seconds by 1 mg/kg. eryth. Sensory endings, nerve conduction and neuromuscular transmission are unimpaired at these concentrations. Spontaneous impulses originating in the central system and led off from peripheral nerve are extinguished with remarkable sudden-

ness by eryth, but are unaffected by dtubo. Electrical recordings from the ventral nerve cord and from roots of the abdominal ganglia show that eryth has no effect on giant-fiber conduction, blocks transmission from giant fiber to root, depresses the spontaneous activity of the cord somewhat, and depresses greatly or blocks the spontaneous activity led off from the root originating presumably in the attached ganglion. Eryth, unlike dtubo, blocks central synaptic transmission in frog and crayfish, and like dtubo blocks neuromuscular transmission in frog.

Anti-stiffness factor in relation to hearing in guinea pigs. ROSALIND WULZEN and ALICE B. PLYMPTON (by invitation). *Dept. of Zoology, Oregon State College, Corvallis, Ore.* 117 guinea pigs were divided into two groups, one on stock diet, the other on diet deficient in anti-stiffness factor. The stock diet consisted of rolled barley, green feed and straw. The deficient diet was composed of skim milk powder and water, plus an adequate supply of minerals and all known vitamins except the anti-stiffness factor. For the test, animals were exposed to signals from an audio-signal generator combined with loud speaker and key. They responded with flicking of the ears to a wide range of auditory stimuli. Only slight deviations were found in repeated readings for any one animal. Limits of normal hearing, judged by the ear flick and expressed as frequency in cycles per second, were established as follows: low range, 200–450, high range, 10000–15000. Of 45 animals on stock diet, 41 proved to have normal hearing; 4 had restricted hearing; none were completely deaf, that is, without ear flick response. Of 72 animals exposed to deficient diet for varying lengths of time, 42 showed normal hearing; 15 had restricted hearing; 15 were completely deaf, that is, without ear flick response.

Fasting serum vitamin A and carotene levels, and vitamin A absorption in males over 40. MARVIN J. YIENGST (by invitation) and NATHAN W. SHOCK. *Section on Cardiovascular Diseases and Gerontology, National Heart Institute, National Insts. of Health, and Baltimore City Hospitals, Baltimore, Md.* The purpose of this investigation was to study age changes in absorption of vitamin A and fasting levels of vitamin A and carotene in a population living under homogeneous conditions. Fasting levels were determined on 121 males ranging in age from 40 to 90 years who were unselected except to exclude any subjects with clinical signs or history of liver disease. All subjects were residents of the Baltimore City Hospitals and Infirmary (Old People's Home) and were receiving an adequate diet. The method of Bessy, Lowry, Brock, and Lopez (*J. Biol. Chem.*, 166, 177 1946) was used in the determinations. The serum vitamin-A levels ranged from 10 to 88 and per 100 ml. with a mean of 49.9. Arranged by decades, the 5 groups from 40 to 90 years of age showed no age differences. The carotene levels ranged from 28 to 231 and per 100 ml. with a mean of 113.1 and likewise showed no significant age difference. A low positive correlation exists between the fasting vitamin A and carotene levels ($r = 0.47$, $N = 121$) in the serum. Fifty-four males included in the above group were fed 100,000 U of vitamin A in oil. Blood samples were drawn at 0, 2, 4, 6, 8, 10, 16, and 24 hours after oral administration. Mean values in and per cent for the group at these times were 53.4, 125.3, 150.2, 172.1, 118.9, 96.7, 73.0, and 63.3. There were no age differences in response to oral administration of vitamin A. Serum carotene values remained unchanged.

Effects of DOCA and adrenal cortical extracts on survival of adrenalectomized rats after burning. S. S. YOU (by invitation) and E. A. SELLERS. *Dept. of Physiology, Univ. of Toronto, Toronto, Canada.* Rats of both sexes weighing about 150 gm. were bilaterally adrenalectomized and maintained on saline for 4–10 days. They were then divided into 3 groups, the first of which constituted a control and continued to receive saline. Desoxycorticosterone acetate and adrenal cortical extract were administered to the second and third groups, which were allowed water ad libitum. A standard burn was produced under ether anesthesia by immersing the backs of all animals in water at 85°C. for 30 seconds. This proved lethal in 2–42 hours to the control group (10 animals). The 28 rats of the second group received DOCA by subcutaneous injection in daily doses of 0.25, 0.50 and 1.0 mg. for 4 to 7 days prior to burning. At the higher dosage levels DOCA afforded a definite protection, 4 of the 5 animals which received 0.5 mg/day for 7 days survived. All the 9 animals receiving 1.0 mg/day for 7 days survived, while in another group of 9 rats which were given the same dosage for 4 days, 2 died. Adrenal cortical extracts from different sources (Connaught and Upjohn Laboratories) were administered to group 3 in doses varying from 15 to 150 dog U/day (divided doses when the dosage was high) for 4–8 days. Among the 24 rats thus treated only 2 animals which received 60 and 150 U/day respectively survived the burning. The effects of these substances on intact animals subjected to a similar stress will also be reported.

A respirator and perfusion pump with new features and an improved constant injection apparatus (motion picture). W. B. YOUMANS. *Dept. of Physiology, Univ. of Oregon Medical School, Portland, Ore.* A piston pump, having new features, and an improved constant injection apparatus have been designed and constructed for this laboratory by the Martin-Hubbard Corp., Boston. The pump will deliver any stroke volume between zero and 1000 cc. A rate continuously

variable from zero to 180/min. is obtained by the use of a Graham transmission unit. Either rate or stroke volume can be changed through the entire range without stopping the motor. There are 4 input ports and one output port. All parts are made of metal, the valves consisting of an axle with sectors cut out to permit passage of the gas at the proper phase of the cycle. The piston empties the cylinder at each downstroke regardless of the stroke volume. A pump having the same basic features with a smaller maximum stroke (500 cc.) which will deliver against pressures in the range of arterial blood pressure is designed also. Use of the instrument to pump gases directly and indirectly and to pump liquids indirectly will be shown. The constant injection apparatus consists of a Graham transmission unit geared to turn a threaded shaft against the plunger of a syringe of any size up to 50 cc. Any injection rate between zero and 10 cc/min. can be obtained without stopping the motor. Uses of the instrument to maintain steady submaximal effects by continuous intravenous injection will be shown.

Bone absorption and relaxation of guinea pig pelvis by prolonged estrogenic injections. WILLIAM C. YOUNG (by invitation) and FREDERICK E. EMERY. *Dept. of Physiology and Pharmacology, Univ. of Arkansas School of Medicine, Little Rock, Ark.* The effects of various estrogens on the pelvis of the guinea pig have been studied in about 60 young guinea pigs of both sexes. The males were injected with 1.0 mg. of diethyl stilbestrol in olive oil for a duration of several months. Periodic X-rays were taken throughout the injection period. By palpation and X-ray it was demonstrated that there was a separation of the pelvis varying from little or no separation to almost complete relaxation in some cases. The testes seemed to be dominated by the estrogens, as shown by the fact that the separation at the pubic symphysis was almost as pronounced in males as in castrated males. Female guinea pigs treated in a similar manner with 0.1 mg. of stilbestrol twice weekly have shown bone absorption and re axation. So far complete disappearance of the bony pelvis, like that of the pocket gopher, has not been obtained.

Response of the young cockerel to methyl folic acid and testosterone propionate. M. X. ZARROW and I. B. KORETSKY (introduced by F. L. HISAW). *Biological Labys., Harvard Univ., Cambridge, Mass.* Studies were made on the relationship of folic acid to the response of the testis and comb of the chick to testosterone propionate. A folic-acid-deficient state was produced by the administration of a crude methyl folic acid preparation (courtesy of Dr. E. L. R. Stokstad, Lederle Laboratories) in the diet. Day-old cockerels of the Leghorn strain were divided into 4 groups of 10 each and treated in the following manner; *1*) normal controls; *2*) 100γ of testosterone propionate daily; *3*) 2% methyl folic acid in the diet; and *4*) 2% methyl folic acid in the diet plus 100γ of testosterone propionate daily. The androgen was dissolved in sesame oil and injected subcutaneously in a daily volume of 0.05 ml. In the first experiment the chicks were treated for 7 days and killed on the 8th day at which time organs were removed for weight determination and histological examination. The testis weights for the 4 groups were 20, 15, 20 and 10 mg. respectively or 27, 20, 28 and 16 when expressed as a function of the body weight. The absolute comb weights were 21, 76, 16 and 54 mg. and the relative weights were 52 98, 39 and 83. A second experiment in which 4% methyl folic acid was used in the diet gave essentially the same results for testis response. The comb weights were 34, 83, 24 and 106 mg. or 40, 90, 37 and 163 respectively when expressed as a function of body weight. These results would seem to indicate that testosterone propionate was able to produce comb growth in the presence of the antagonist.

Effect of adrenoxyl on blood loss from surgical wounds. J. J. ZAVERTNIK and R. F. HAGERTY (introduced by K. S. GRIMSON). *Dept. of Surgery, Duke Univ., Durham, N. C.* Adrenoxyl, a monsemicarbazone of adrenochrome, reportedly decreases bleeding time of rabbits and man. In our studies in dogs it was determined that intramuscular injection of 10 gamma of Adrenoxyl does produce a marked decrease, effect being maximal by 30 to 60 minutes. A study of ability of the drug to decrease blood loss from surgical wounds was also undertaken. Consecutive apparently similar incisions were made in the liver of dogs estimating bleeding by weight of blood collected. One hour after administration of 10 gamma of Adrenoxyl amount of bleeding decreased markedly. Wounds were also produced by resection of similar portions of a rabbit's two ears. Amount of blood loss was found decreased moderately one hour after administration of 10 gamma of Adrenoxyl. Three similar wounds were produced on opposite ears of the same rabbit 5 days apart. The 3 different wounds produced on one ear before the drug bled respectively an average of .31 gm., 72 gm. and 80 gm. of hemoglobin, increasing amounts representing the larger or more proximal incisions. Five days later 3 similar wounds were made after Adrenoxyl using the opposite ear. Decrease in bleeding after administration of Adrenoxyl was 44.7%, 3.7%, and 13.3% respectively. Decrease after Adrenoxyl was greater in wounds where bleeding occurred predominantly from small vessels. A less marked decrease occurred from 3 wounds of control rabbits produced 5 days after incisions of the opposite ear and without using Adrenoxyl. Hematologic examinations demonstrated a rise in the platelet count which might explain this observation.

Effect of low protein and plasmapheresis on fluid compartments and glomerular filtration rate. MARJORIE B. ZUCKER, LOUIS CIZEK (by invitation) and DOUGLAS TOMPKINS (by invitation). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* Weekly measurements were made on 3 dogs on a control diet of Beacon Meal alone, and then after 3 weeks on a diet supplying 1.6 gm. protein/kg/day and 80 Cal/kg/day (50% Beacon Meal, 16% dextrose, 33% Mazola, 0.5% NcCl, 0.2% yeast, 0.4 gm/kg/day Cal-C-Tose, 0.4 drops/kg/day oleum percomorph). Finally, determinations were made for several weeks during which the diet was continued and the dogs plasmapheresed 1 to 4 times. Including a weekly supplement, the NaCl intake approximated 20 mEq/kg/week. Clinical edema was not observed. The first number in following parentheses represents percentage change from the control value in dog 1, the second in dog 2, etc. The diet alone caused weight loss (−17, −13, −4), decreased serum protein concentration (−10, −6, −10), electrophoretic A/G ratio (—, −49, −34), colloid osmotic pressure (COP) (−8, −13, −16), T-1824 plasma volume (PV) (−23, −23, −26), creatinine clearance (GFR) (−26, −30, −46) and, in 2 dogs, reduced thiocyanate space (AF) (−21, −23, —). The latter is contrary to Keys' results in man on a famine diet. The results after plasmapheresis, expressed as percentage change from the values observed on the low protein diet, are given for the week during which the lowest COP was observed, and were determined at least 2 days after bleeding. Plasmapheresis did not alter the weight (−3, 0, 0), but usually reduced the serum proteins (−16, —, −11), A/G (—, +10, −34) and COP (−31, −32, −27). PV increased (+10, +5, +23), a finding which is difficult to explain. The maintenance of the GFR at a low level (−10, −15, +3), is of interest. AF increased (+12, +16, —), indicating that fluid retention occurred although COP was not reduced to values associated with clinical edema.

Hepato-renal factors in circulatory homeostasis. XXVI. Effect of adrenalectomy on renal VEM system and hypertension. BENJAMIN W. ZWEIFACH and EPHRAIM SHORR. *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* The formation of a vasoexcitor principle (VEM) by kidney appears to depend on the presence of the adrenal cortex, kidneys from adrenalectomized animals failing to produce VEM *in vitro*. The present study deals with attempts to produce renal hypertension in adrenalectomized rats maintained on a high salt diet in apparently good health for periods of 30 to 120 days. Various methods for inducing hypertension were employed; capping of one or both kidneys with gauze-collodion, partial ligation of the renal artery, or figure-eight loop around the kidney. These experimental procedures, which produced hypertension in about 75 to 85% of the control rats, did not induce any clear-cut hypertension in a comparable series of 45 adrenalectomized rats maintained on salt. Microscopic observations on the mesoappendix of these latter animals showed no vascular hyperreactivity such as occurs in the terminal arterioles and precapillaries of hypertensive rats. There was also no hypertrophy of the blood vessels of the mesenteric capillary bed such as we have found to occur during the development of hypertension in rats. A previous study has shown that kidneys of hypertensive animals develop a metabolic lesion as a result of which VEM production, normally limited to anaerobiosis, now takes place aerobically as well as anaerobically. No such metabolic alteration was present in the kidneys of the adrenalectomized rats in this study. The relation of the content of renin in such kidneys and of the α_2-globulin substrate in the blood of these animals will be discussed.

Muscular performance during positive radial acceleration. LAURENCE E. MOREHOUSE. *Dept. of Aviation Medicine, School of Medicine, Univ. of Southern California, Los Angeles, Calif.* Performance of pilots in a simulated cockpit on a human centrifuge was photographed by a motor driven high speed movie camera. Deviations in normal performance were studied. During acceleration, even at low levels, errors were repeated by trained subjects which would have marked effect on aircraft control. Physiological measurements of alterations in the functions of the nervous and muscular systems during positive radial acceleration indicate that, even at blackout g levels, normal function is intact. Errors in neuromuscular control, best shown by electromyographic records, suggested that alterations in proprioceptive sensations induced by g forces acting upon the moving segments played the major role in affecting performance.

Action potentials in single muscle fibers. W. L. NASTUK and A. L. HODGKIN (introduced by M. I. GREGERSEN). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City, and the Physiological Laboratory, Cambridge, England.* Graham and Gerard (*J. Cell. & Comp. Physiol.* 28: 99, 1946) and Ling (*Federation Proc.* 7: 72, 1948) showed that the potential difference across the surface membrane of a resting muscle fiber could be measured directly by transverse impalement of the cell with a microelectrode. We have repeated these experiments using equipment capable of recording both action and resting potentials. Frog sartorius muscle, stretched in order to minimize movement, was stimulated either through its motor nerve or directly. Deflections in the record attributable to contraction were

often seen, but they did not appear until after the spike and under favorable circumstances might be absent altogether. Most of the experiments were carried out with electrodes which were filled with 3M KCl, but results qualitatively similar were obtained with electrodes filled with 0.118M KCl, but results qualitatively similar were obtained with electrodes filled with 0.118M KCl or with Ringer's solution. Electrodes filled with 3M KCl are believed to give the most accurate results since *1*) junction potentials should be small, *2*) electrode resistances are relatively low, *3*) the quantity of KCl diffusing from the electrode during the time required for measurement can be shown to be negligible. In experiments at both 19°C. and 7°C. it was found that the action potential exceeded the resting potential in magnitude. It has been suggested that the membrane potential is reversed during activity because the membrane becomes selectively permeable to sodium during that period. If this is correct, one would expect that the membrane would cease to reverse its potential during activity if the sodium concentration in the external fluid is reduced to a low value. We have found that the resting potential and the action potential are approximately equal when the external sodium concentration is lowered to 30% of normal, and that over a considerable range the magnitude of the action potential is proportional to the logarithm of the external sodium concentration.

Adenosine phosphorylation by rabbit kidney homogenate. DAVID RAPPORT, ATTILIO CANZANELLI, and RUTH GUILD (by invitation). *Dept. of Physiology, Tufts College Medical School, Boston, Mass.* Rabbit kidney was homogenized in KCl-$NaHCO_3$, and to the mixture was added $MgCl_2$ or SO_4 (0.01M), Na Succinate (0.01M), Na F (0.01M), and Sorensen phosphate buffer, (0.02M), the molarities representing final concentration. The final tissue dilution was 1:4. This was incubated aerobically alone or with added adenosine in final concentration of 0.03M, usually for 30 min. at 37°. Phosphate and pentose were determined in the acid filtrate and in the barium and alcohol insoluble fractions. The evidence indicates that under our conditions adenosine, or one of its products, was phosphorylated, and that in the course of this phosphorylation, pentose disappeared. The resulting phosphate ester was not a mononucleotide, such as was reported by Colowick, Kalckar and Cori (*J. Biol. Chem.* 137: 343, 1941). The failure to find adenylic acid indicates that animal tissue behaves differently in this respect than yeast (Ostern and Tersakowee, *Zeitschr. f. physiol. Chem.* 250: 155, 1937). The results suggest that a mechanism similar to that described by Schlenk and Waldfogel (*Arch. Biochem.* 12: 181, 1947) may be operating.

An enzymatic reaction involving amino acids and adenosine triphosphate. JOHN M. REINER. *Dept. of Physiology, Tufts College Medical School, Boston, Mass.* In the search for an intermediate of peptide bond formation, the direct phosphorylation of amino acids by adenosine triphosphate was attempted. When a rat liver homogenate in isotonic KCl is incubated for 15 minutes at 37°C. with 10 micromoles of adenosine triphosphate and 10 to 20 micromoles of amino acid, a labile phosphate compound is formed. With more prolonged incubation, the labile phosphate decreases, appearing as inorganic phosphate. A washed 'mitochondrial' fraction of the homogenate contains the activity; requires as a cofactor $MnSO_4$, the optimal concentration being 0.0016M, and inorganic phosphate. No labile phosphate is formed in the absence of amino acid, in the absence of adenosine triphosphate, or in the presence of only catalytic amounts of the latter. Activity has been obtained with glycine, methionine, arginine, histidine, tryptophane, and aspartic acid. The activity increases with increasing amounts of enzyme and of adenosine triphosphate. Activity is inhibited by 2,4-dinitrophenol. The product is extremely labile: at *p*H 4 it is destroyed in 30 minutes at room temperature, and the hydrolysis is accelerated by molybdate; cold 5% trichloroacetic acid splits it within a minute or two, so that deproteinization must be performed with saturated ammonium sulphate. Negative results have been obtained with Lipmann's hydroxylamine reagent for acyl phosphates. This suggests that an N-phosphoamino acid may be involved; however, since it is not known how the adjacent amino group would affect the reaction of an acyl phosphate with hydroxylamine and the color formation with ferric ion, the alternative possibility cannot be ruled out.

A new blood supply for ischemic hearts, by connecting the aorta with the coronary vessels. JOSEPH THOMAS ROBERTS. *Univ. of Buffalo School of Medicine, Buffalo and Veterans Adm. Hospital, Batavia, N. Y.* The concept of revascularizing ischemic hearts by bringing aortic blood to the myocardium through the coronary sinus and veins was introduced earlier by the author. Since then others have confirmed his report that such revascularization inhibited myocardial infarction after ligation of the coronary arteries. This operation devised by the present author is now being applied to human patients suffering from myocardial ischemia. Subsequent studies on the procedure are brought up to date, as are efforts to anastamose the internal mammary artery with the coronary arteries and veins. It is predicted that this operation may become a useful method of treating the coronary artery disease problem. After such anastamosis, myocardial blood is apparently drained by the Thebesian vessels as well as by other routes.

Initial effects of potassium on the mechanical responses of skeletal muscle. ALEXANDER SANDOW and ARTHUR J. KAHN (by invitation). *Washington Square College of Arts and Sciences, New York Univ., New York City.* The present studies (supported by a grant from the Office of Naval Research under Contract N6onr-279, Task order 7) deal with changes in the latency relaxation (LR) and peak tension (T) of isometric twitches of the frog sartorius immediately after immersion of the muscle in K-enriched Ringer's solution. Square-wave, massive, transverse shocks, applied to the muscle immersed in the test solution, are used as stimuli. The LR is recorded by the piezoelectric, cathode-ray method, and T by optical myography. During an initial period of 3 to 4 minutes after placing a muscle in a solution containing 28 mg. % K, both the magnitude (R) of the LR and T increase. The alterations are reversed relatively slowly after the initial period, although T does so somewhat more slowly than R. At the end of the initial period, the maximal increases, when compared with responses in normal Ringer's (7.8 mg. % K), are approximately R, 100% and T, 25%. Solutions containing some 50 mg. % K or greater cause only immediate decreases in R and T, although initial increases, too fleeting to observe, may have occurred. The initial increases observed with the smaller potassium concentrations develop so rapidly, before any appreciable K had accumulated intracellularly, that it is inferred the added K could only have directly affected the behavior of the membrane excitatory mechanism. This view will be discussed in relation to the significance of the LR in the process by which excitation and contraction are linked in the response of the muscle fiber.

Action potentials of giant fibers of the crayfish: cambarus clarkii. C. A. G. WIERSMA. *Kerckhoff Labys. of Biology, California Inst. of Technology, Pasadena, Calif.* Action potentials lead off from partially or totally isolated giant fibers of the central nervous system almost invariably show instead of a single phase under one electrode, a double phased deflection. The first phase is always positive, and depending on leading off conditions, varies in size from very small to as large as the subsequent negative deflection. All trials to account for this positive deflection as an artifact or a pick up of a distant potential by the inactive electrode, have thus far failed. Especially convincing for the independent nature of the positive deflection which occurs under the active electrode were experiments in which both leads were taken from a part of an isolated fiber, completely surrounded by oil. Under these circumstances there will be at first a diphasic potential with a monophasic lead (quadruple phasic with bipolar leads). After a short time in oil the negative phase becomes smaller and disappears, leaving a large positive variation. On resubmerging of the fiber in physiological fluid and subsequent return to oil this order of events can be repeated several times. In fiber bundles a diphasic deflection remains under these circumstances, only the isolated giant fibers show this phenomenon. As a working hypothesis it may be supposed that these fibers contain two double layers of opposite sign. The inner, which would be responsible for the positive deflections, would use the very fluid interior axoplasma as a conductive medium and would hence not be influenced by the increase in outside resistance caused by oil

THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC.

Fortieth Annual Meeting

DETROIT, MICHIGAN, April 18–22, 1949

(For possible corrections in any of the following abstracts see the June issue)

Phenolsulfatase of animal tissues. LYNN D. ABBOTT, JR. and MARY K. EAST (by invitation). *Dept. of Biochemistry, Medical College of Virginia, Richmond, Va.* In order to establish more precisely the optimal conditions for the determination of animal phenolsulfatase activity, we have determined *p*H-activity curves with rat liver and kidneys and temperature-activity curves with rat liver using *p*-nitrophenylsulfate as substrate. The optimum *p*H appears to be slightly higher (about 6.6) than that of the phenolsulfatase of takadiastase (6.2). As was previously noted with the takadiastase enzyme, the rate of reaction was much greater at 50° than at 37°C. We have studied the phenolsulfatase activity of the sera and livers of normal rats, carbon tetrachloride-poisoned rats, and rats exposed to CCl_4 but protected by xanthine injection. The data indicate that the serum phenolsulfatase activity of carbon tetrachloride-poisoned rats was greater than that of control animals or of protected animals. The phenolsulfatase activity of the liver of carbon tetrachloride-poisoned rats appeared to be decreased.

Effect of uric acid on the peroxidative detoxification of diphtheria toxin. KJELL AGNER (introduced by J. W. WILLIAMS). *Dept. of Chemistry, Univ. of Wisconsin, Madison, Wis.* It has been previously reported (*Fed. Proc.* 7: 140, 1948) that crude diphtheria toxin preparations are detoxified by peroxidases in the presence of H_2O_2. Purified toxin was not detoxified in experiments performed under similar conditions. The reaction required the presence of a dialyzable substance or substances present in the crude culture filtrate of diphtheria bacilli. It has now been found that the above crude filtrate can be replaced by a dilute solution of uric acid. Verdoperoxidase as well as horse radish peroxidase oxidize uric acid in the presence of low concentrations of H_2O_2. The rate of the destruction of uric acid is increased by the addition of diphtheria toxin. It seems likely that an intermediate oxidation product of uric acid reacts with the toxin to give detoxification.

Antimycin antibiotics. KAMALUDDIN AHMAD (by invitation), F. MERLIN BUMPUS (by invitation), B. R. DUNSHEE (by invitation) and F. M. STRONG. *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* A new antibiotic, antimycin A, has been isolated from cultures of a *Streptomyces* species. Antimycin A is a nitrogenous phenol of the probable formula $C_{28}H_{40}O_9N_2$. Alkaline hydrolysis destroys the antibiotic activity, and gives rise to a nitrogenous phenolic acid, a neutral, nitrogen-free product, and two moles of a volatile fatty acid. Antimycin A is highly toxic to many yeasts and other fungi, but is relatively inactive toward bacteria. Its effect on several of the more sensitive organisms is easily observable at concentrations of a few parts per billion. It inhibits the respiration of *Saccharomyces cerevisae* Y30 and stimulates aerobic fermentation up to the anaerobic rate. It also inhibits the cytochrome oxidase and succinic dehydrogenase activities of rat liver homogenates at concentrations in the range of 10^{-6} molar. Paper chromatography of crude extracts of the original culture filtrate reveals the presence of two additional antibiotics, provisionally designated as Antimycin B and C.

Abnormal excretion of tryptophane-like substances in tuberculosis. ANTHONY A. ALBANESE, L. EMMETT HOLT, JR., MARILYN LEIN (by invitation), EMILIE M. SMETAK (by invitation) and BETTY VESTAL (by invitation). *Dept. of Pediatrics, New York Univ. College of Medicine, New York City.* By the application of the tryptophane and amino N methods developed by some of us to the urines of patients of both sexes the following data were obtained:

Clin. diag.	Age group	No. of det.	Tryptophane amino N / Total amino N; %
Non-tubercular	3 m.–1.5 yr.	55	2.6 ±1.0
	2–14 yr.	20	3.4 ±0.7
	18–40 yr.	48	4.5 ±0.6
Tubercular	3–13 yr.	32	6.7 ±1.0
	20–62 yr.	9	7.1 ±0.8

Successive measurements on patients over a period of 2–3 years gave the impression that *a*) the T.A.N./A.N. ratios are consistently higher for chronic than for acute forms of tuberculosis; *b*) critical episodes are characterized by sharp increases in the ratio, whereas recovery is accompanied by a decrease in this value; and *c*) the abnormal T.A.N./A.N. ratios are not due to febrile

states. Comparable T.A.N./A.N. ratios derived from urinary tryptophane figures obtained by the microbiological or Shaw-McFarlane techniques did not yield differentiating values for the tuberculosis patient as found by the Albanese-Frankston method. Moreover the high ratios do not arise from an increased output by the tubercular subject of a non-diffusible bound N component. This however does not exclude a possible increase in the output of diffusible bound N. The metabolic and nutritional implications of these observations will be discussed.

Biological value of wheat and corn proteins in the male infant. ANTHONY A. ALBANESE, L. EMMETT HOLT, JR., SELMA E. SNYDERMAN (by invitation), MARILYN LEIN (by invitation), EMILIE M. SMETAK (by invitation) and BETTY VESTAL (by invitation). *Dept. of Pediatrics, New York Univ. College of Medicine, New York City.* During the past year we have made measurements on the food value of some vegetable proteins in the infant. We felt this worthwhile for two reasons; *a*) vegetable protein containing diets might prove useful in feeding infants allergic to animal protein diets and; *b*) the use of vegetable protein diets might prove necessary if predictions regarding the failure of future food production to keep pace with population growth are realized. A synthetic diet containing lysine reinforced granulated wheat gluten (4% lysine content) as the principal N component was found to support N retention, body weight gains and blood protein concentration in 3 male infants equal to those achieved with an evaporated milk formula fed at comparable N, caloric and fluid levels. On the other hand a similar diet fed under similar conditions in which corn gluten reinforced with 6% L-lysine and 1% L-tryptophane constituted the principal protein component was found not to support N retention and weight gains at levels comparable to those attained with the evaporated milk formula. The inferior biological value of the corn gluten diet was not due to poor digestability which we had previously found to be cause of the poor nutritional quality of commercial zein. In the light of recent investigations on the effects of amino acid inbalance on the growth of experimental animals reported from various laboratories we venture to suggest that the inferior nutritional quality of reinforced corn gluten in the infant may arise in part from its failure to provide an amino acid pattern compatible with growth.

Isolation of adenosine triphosphate from plant tissue. H. G. ALBAUM, M. OGUR (by invitation), and A. HIRSHFELD (by invitation). *Depts. of Biology and Chemistry of Brooklyn College, Brooklyn, N. Y.* We have previously reported (*Fed. Proc.* 7: 141, 1948) an adenine-pentose-pyrophosphate in trichloracetic acid extracts of the mung bean. The isolation of adenosine triphosphate (ATP) from a plant source, seemed a necessary experimental step in the extension of common pathways of metabolism to plant tissues. The present work deals with the isolation of the plant ATP with a minimum purity of 70% when calculated as $Ba_2ATP \cdot 4H_2O$. A trichloracetic acid extract of mung bean sprouts was fractionated with barium into two soluble fractions (*p*H 4.5–8, and *p*H 1–4.5), and a residue insoluble at *p*H 1. Approximately 80% of the material showing ultraviolet absorption at 260 mμ. was found in the fraction insoluble at *p*H 1. This material, which may be dissolved and freed of barium by sodium sulfate exchange, represents an ATP extract of approximately 10% purity. Precipitation of the silver salt, decomposition with HCl, and reprecipitation of the barium salt increased the purity to approximately 50%. This barium salt is pigmented and shows absorption maxima at 320 mμ. as well as at 260 mμ. Removal of the barium by ion exchange, using Amberlite IR 100, and treatment with four volumes of acetone yielded a white barium salt with no absorption at 320 mμ. This preparation has a ratio of pentose:adenine:labile P:total P of 1:1:2:3 and a minimum purity of 70%. It is active in the transphosphorylation of glucose by yeast hexokinase. After treatment with myokinase and hexokinase, it is deaminated by a muscle deaminase at a slow rate compared with animal ATP.

In vitro inhibition of hyaluronidase activity. HARVEY E. ALBURN, ROBERT W. WHITLEY and LOUISE C. HALL (introduced by SAM SEIFTER). *Wyeth Institute of Applied Biochemistry, Philadelphia, Pa.* In order to study the nature and amount of hyaluronidase inhibitor present in hyaluronic acid preparations, the inhibition by heparin and other sulfated polyuronides and the neutralization of this inhibition by salts and protamine were investigated. The hyaluronidase activity was determined by a modification of the Kass and Seastone turbidimetric method and a highly purified hyaluronic acid from human umbilical cord was used as the substrate. With 0.1 mg. of hyaluronate and sufficient hyaluronidase to hydrolyze it almost completely in 1 ml. of *p*H 6.0, 0.1 M potassium acetate buffer in 30 min., 0.0033 gamma of heparin gave detectable inhibition and 0.33 gamma gave maximal inhibition in the absence of sodium chloride. In the presence of 0.075M sodium chloride, the optimal concentration, 10 gamma of heparin was required to give detectable inhibition. Similar data were obtained for other salts and other polyuronides. These data were used to calculate the amounts of inhibitor in various hyaluronate preparations. Protamine was found to neutralize the inhibition of hyaluronidase by heparin in very low concentrations, probably due to combination with the heparin. In higher concentrations protamine was found to inhibit hyaluronidase activity.

Effects of 2-acetylaminofluorene on liver func-

tion. JAMES B. ALLISON, ARTHUR W. WASE (by invitation) and WALTER W. WAINIO (by invitation). *Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.* Studies are underway on the effects of the carcinogenic agent, 2-acetylaminofluorene, on liver function in dogs and rats. The animals are being fed a synthetic diet (*J. Nutrition* 29: 413, 1945) consisting of casein 12%, lard, carbohydrate, agar, minerals, synthetic vitamins and 0.03 to 0.05% 2-acetylaminofluorene. Preliminary results on the dogs demonstrate that after 4 months subsistance on this diet the excretion of uric acid increased markedly while the excretion of allantoin decreased; changes which have been associated with decreased uricase activity in damaged livers. Decreased liver function was indicated also by an increased retention of bromsulfalein, and by jaundice. At the end of 6 months a hepatoma had developed which was sufficiently advanced in 1 dog to cause death. The neoplasm had spread also to the spleen in this animal. The correlation between these and other metabolic changes associated with the development of hepatomas are being followed together with the retention of nitrogen in the body of the animal.

Administration of parenteral and oral casein hydrolysates to depleted dogs. CARL ALPER (by invitation), BACON F. CHOW and SHIRLEY Q. DEBIASE (by invitation). *Nutrition Dept., Div. of Development, and the Div. of Protein Chemistry, The Squibb Institute for Medical Research, New Brunswick, N. J.* Mongrel dogs, previously depleted by feeding a protein-free diet at normal caloric intake, with or without the accompaniment of plasmapheresis, were fed parenterally for a period of four weeks, and then orally for a similar period, a tryptic digest of casein as the sole source of nitrogen at a level sufficient to maintain positive nitrogen balance. The object of this experiment was to study the effectiveness of parenterally and orally administered casein hydrolysates in the repletion of plasma protein, and the maintenance of positive N balance. Maintenance of N balance, and the repletion of plasma protein were measured by urine, fecal, and blood protein N determinations and blood-volume determinations. Results of these experiments, which were carried out in 10 dogs, showed: *1*) no striking increments in weight during repletion; and *2*) dogs retained 2 to 3-fold more N via the oral route than via the intravenous route, even though partial repletion had already been effected by the intravenous route. Also it was observed that, contrary to the current belief, the degree of hydrolysis varying from 18 to 40% did not appear to influence the utilization of N. Between 5–9% of the N retained by parenteral feeding was utilized for plasma protein synthesis. About 1.5–2.5% of the N retained by oral feeding was utilized for plasma protein synthesis.

Microbially synthesized APF (animal protein factor). STEFAN ANSBACHER, HARLEY H. HILL, JR. (by invitation), JESSE W. TIEMAN (by invitation), JEAN F. DOWNING (by invitation) and JOHN H. CALDWELL, JR. (by invitation). *Research Laboratory, Vi-D-Co., Marion, Ind.* The need of single-stomached animals for APF appears to increase when increasing amounts of soybean oil meal (SBOM) are fed. It would seem logical, therefore, to impart APF potency to SBOM. This has been accomplished by growing certain micro-organisms on media, the principal constituent of which was SBOM. Yeast, although an excellent source of the vitamin B-complex, has normally no APF activity, but it too may be endowed with high APF potency by certain fermentation processes. The micro-organisms that selectively synthesize APF have been isolated for use in these processes. The physiological activity is expressed in APF units. Fish meal, fed to chicks at a level of 5% of the basal (all-plant) ration, has been assigned a potency of one unit. If a product fed at the same level causes chicks to grow approximately as well as those receiving the fish meal ration, it has a potency of one unit. The potency is two units, if the oral administration of a product at 2½% results in growth about equal to that obtained from the fish meal ration. Products containing at least 20 APF units have been obtained by fermentation processes without resorting to chemical purification procedures.

Chromatography of urinary steroids and products of acid hydrolysis of pure steroids. R. M. ARCHIBALD. *Hospital of the Rockefeller Institute for Medical Research, New York City.* CCl_4 extracts of hydrolysates of 10 to 50 cc. urine have been chromatographed on columns in a Technicon fraction cutter. Eluting agents used were benzene, benzene plus 0.1%, 0.5%, and 2.0% ethyl alcohol, 95% ethyl alcohol, water, and 50% pyridine in water. Ultraviolet absorptions at 215, 240, 260 mμ. were determined on each of ninety 3 cc. fractions; then aliquots of each were used for Zimmermann, Liebermann-Burchard and modified Kober (color and fluorescence) reactions, and the colorimetric method of Munson, *et al.* for dehydroisoandrosterone. By these methods comparisons were made of chromatograph fractions of extracts obtained after hydrolysis of pure steroids and urines with HCl, H_3PO_4, H_2SO_4, or NaOH *a*) at room temperature *b*) after reflux boiling 30 minutes. Application of these techniques to urine of a woman before and after surgical removal of a hirsutizing adrenal tumor, and to an extract of the tumor, indicated the presence of a large amount of material easily eluted with benzene in the tumor and in the urine prior to operation, but absent from urine after operation.

Determination of dimethylethanolamine in biological materials. CAMILLO ARTOM and MARIETTA CROWDER (by invitation). *Dept. of Biochemistry,*

Bowman Gray School of Medicine, Winston-Salem, N. C. The method is based on the observation that dimethylethanolamine (DME), a likely intermediate in choline metabolism, is rapidly and quantitatively displaced from its alkaline solutions by steam distillation. The protein-free material is treated with HNO_2 to destroy primary and secondary amines, transferred to the distilling flask of the Parnas-Wagner apparatus, brought to *p*H 10, and aerated at room temperature to remove volatile tertiary amines. DME is then steam distilled for 15 minutes into an excess of standard acid and the excess acid titrated iodometrically with 0.005 N $Na_2S_2O_3$. 95 to 100% of the substance in pure solutions or added to tissue extracts could be recovered. Choline, ethanolamine, methylethanolamine, serine, urea, glycine, betaine or trimethylamine do not interfere appreciably. The method has been applied to aqueous extracts of liver and muscle, deproteinized with colloidal $Fe(OH)_3$, and to acid hydrolysates of the lipides, extracted from the tissues of rats under various dietary conditions. Traces of DME were found only occasionally, the results being negative in most determinations. However, quite definite amounts of DME were detected in the liver lipides of rats, killed 3 or 6 hours after the administration of a single large dose of the compound. This finding indicates that DME can be incorporated in the phospholipide molecule even before further methylation to choline. Determinations of DME in the tissues of animals receiving large doses of choline or ethanolamine are in progress.

Response of pteroylglutamic acid (PGA) depleted rats to supplementation with natural products. CONRADO F. ASENJO. *Dept. of Chemistry and Nutrition, School of Tropical Medicine, San Juan, Puerto Rico.* PGA-depleted rats, 7 weeks old, were continued on the highly purified PGA-free diet, containing 2% succinylsulfathiazole, but, in addition, were given daily weight amounts of natural products for a period of 5 consecutive weeks. The amount of the supplement was never more than 2 gm., so as not to interfere to any great extent with the basal diet intake. Two % by weight of SST was mixed with the daily supplement. Growth response, white cell regeneration, degree of alopecia, and spleen condition were recorded at the end of the supplementation period. As the depleted rat requirement of PGA to regain normalcy is approximately known (*J. Nutrition*, 36: 601, 1948), the response exhibited to the different supplements by the depleted animals can be evaluated roughly in terms of PGA. Of 10 fresh tropical fruits so far assayed, the only one to exhibit appreciable curative action on the rat was the avocado. Boiled and dried pigeon peas, red kidney beans, and breadfruit seed proved to be rich sources of PGA, inducing very rapid and pronounced recovery. In the present report the results obtained with 20 natural products commonly used in the West Indian dietary, especially tropical products, are presented.

Action of ribonuclease on ribonucleic acid. JAMES E. BACHER (by invitation) and FRANK WORTHINGTON ALLEN. *Div. of Biochemistry, Univ. of California Medical School, Berkeley, Calif.* Ribonucleic acid from yeast was purified and dialyzed; neutralized to *p*H 7.0 and subjected to ribonuclease action. Dialysis was continued and the dialyzable and non-dialyzable fractions were isolated by lyophilization. Control experiments without enzyme were treated similarly. Application of rate studies to fractions that were isolated gave the following data.

	Ribonucleic Acid	Control Na salt	Dialyzable Fraction	Non-dialyzable Fraction
	%	%	%	%
Total N	15.4	13.7	12.3	13.7
Guanine N / Total N	34.3	34.9	24.6	39.2
Purine N / Total N	67.9	67.4	53.8	73.0
Total P	9.0	8.2	8.3	7.8
Labile P / Total P	50.6	51.2	34.6	57.3
Stable P / Total P	49.4	48.8	65.4	42.7
	atoms	*atoms*	*atoms*	*atoms*
Purine N / Labile P	5.06	4.89	5.11	4.98
Pyrimidine N / Stable P	2.44	2.48	2.32	2.47

Ribonucleic acid contains approximately equivalent quantities of each nitrogenous constituent. After ribonuclease action the dialyzable fraction, which comprises 25%, contains 2 pyrimidines per purine, more adenine than guanine and approximately twice as much uracil as cytosine. The non-dialyzable fraction, which comprises 70%, reflects the contrary.

Transaminase activity in the skeletal muscles of normal and vitamin E-deficient animals. MARY ALICE BARBER (by invitation), DANIEL H. BASINSKI (by invitation) and H. A. MATTILL. *Dept. of Biochemistry, State Univ. of Iowa, Iowa City, Iowa.* To examine further into the altered energy metabolism accompanying the dystrophy of vitamin E deficiency, aspartic α-ketoglutaric transaminase activity was investigated in skeletal muscle homogenates from normal and E-deficient guinea pigs and rabbits. In dystrophy, this trans-

amination reaction was decreased by ½ in guinea pigs and by ⅓ in rabbits. The diminution of activity was consistent whether the results were calculated on the basis of wet weight of the tissue or on the dry weight or N content of the homogenate. The concentration of the coenzyme, pyridoxal phosphate, appeared not to be the limiting factor. The reduced activity was not an artifact produced by altered removal of the oxalacetic acid formed. On the contrary, normal muscle seemed to possess a better alternative mechanism for disposing of excess oxalacetate than did dystrophic muscle. The significance of these results in the interpretation of the abnormal energy metabolism of these paralyzed muscles is briefly discussed.

Intracellular heterogeneity of liver ribonucleic acid as evidenced by P^{32} uptake. CYRUS P. BARNUM and ROBERT A. HUSEBY (by invitation). *Depts. of Physiological Chemistry and Physiology, Univ. of Minnesota Medical School, Minneapolis, Minn.* From fasted, homogenized mouse livers ribonucleic acid (RNA) has been isolated from the nuclei (N-RNA), the cytoplasmic particulate fractions (M-RNA), and the cytoplasmic fraction that does not sediment in 90 minutes at 23,000 *g* (MS-RNA). The mice had been injected intraperitoneally with inorganic P^{32} at times varying from 15 minutes to 24 hours prior to sacrifice. The N-RNA shows a specific activity (S.A.-disintegrations/min/γ total P) which rises rapidly so that at 1 hour it is 14% of the S.A. of tissue inorganic P (I.P.) as compared to cytoplasmic phospholipid P for which the S.A. is 10.5% that of I.P. The S.A. of N-RNA passes through a maximum around 3 hours and is below that of phospholipid at 6 hours. The M-RNA takes up P^{32} very slowly and at 1 hour its S.A. is only about 0.6% that of N-RNA but rises to 3.1% at 3 hours and 21.5% at 20 hours. No significant differences have been observed in the S.A. of M-RNA samples obtained from mitochondria (sedimented in 5 minutes at 23,000 *g*) or microsomes (sedimented in 90 minutes at 23,000 *g*). The MS-RNA follows a S.A.-time curve intermediate between those of N-RNA and M-RNA. At 1 hour its S.A. is about 6.8% that of N-RNA while at 3 hours it is 9.1% and at 20 hours 30.5%. These data point up the relationship between intracellular location of RNA and its rate of incorporation of newly administered inorganic phosphate.

Anterior pituitary growth hormone preparations and the glutamine-glutaminase system. PAUL D. BARTLETT (introduced by OLIVER H. GAEBLER). *Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.* Van Slyke *et al* (*J. Biol. Chem.* 150: 481, 1943) have shown that transport and storage of ammonia are physiological functions of glutamine. Storage of amino nitrogen might be another function, and, in this respect, glutamine might be considered a major constituent of Schoenheimer's 'metabolic pool' of nitrogen. Since in this and other laboratories weight gain and nitrogen storage have been produced in rats and dogs with growth hormone preparations it seemed of interest to study the *in vivo* effect of the recent Fishman, Wilhelmi, Russell preparation on the glutamine-glutaminase system. In 19, 20, and 28 day experiments on dogs 3 different lots of this preparation were tested. Increases in plasma glutamine of 19, 30, and 33% above the average control level were obtained. Nitrogen storage and weight gain were produced and excretion of urinary ammonia was found to parallel changes in plasma glutamine. Experiments now in progress will determine whether or not the increase in plasma glutamine occurs independently of total free amino acids. In *ad libitum* feeding experiments on hypophysectomized rats and on hypophysectomized rats treated with growth hormone glutaminase assays of 21 and 31 U/ml. of 25% kidney emulsion respectively were obtained. Food consumption of treated rats did not increase during the 7-day experimental period. Kidneys from normal rats maintained on a food intake based on that of the hypophysectomized-treated rats and from normal rats fed *ad libitum* assayed respectively 35 and 56 U/ml. of 25% emulsion.

Behavior of the thyroid toward elements of the seventh periodic group. II. Rhenium. EMIL J. BAUMANN, N. ZIZMER (by invitation), ELEANOR OSHRY (by invitation) and S. M. SEIDLIN (by invitation). *Laboratory Div. and Laboratory of Medical Physics, Montefiore Hospital, New York City.* Two years ago we presented experiments to the Society which showed that the thyroid filters not only I from the circulation but other halogens as well. We expressed the view that the thyroid distinguishes between the halogens imperfectly if at all in the filtration process. Further, it was suggested that if a very large amount of one of the halogens were administered, compared to the amount of any of the other halogens ingested, the one given in excess will tend to wash the others out of the thyroid in a mass action-like effect. On the basis of other work, we postulated then that other elements of group VII of the Periodic Table would behave as the halogens do and we now report our experiments with Rhenium (element 75). Perrhenic acid given parenterally to rats and rabbits was found to accumulate in the thyroid in greater concentration than in any other tissue—25 to over 100 times more than in the tissue having the next highest concentration. The maximum concentration occurs 1 to 4 hours after injection. Most of the Re is excreted in the urine during the first day and the remainder is practically all eliminated in the next 24-hour period.

Effects of glucose, bile salts, and protein on re-

covery of neutral 17-ketosteroids. WILLIAM T. BEHER (by invitation) and O. H. GAEBLER. *Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.* Rates of color development of individual 17-ketosteroids, and effects of varying other conditions in the Zimmermann reaction, have been studied extensively (*Endocrinology* 33: 189). Little attention has been given to effects of normal and pathological constituents of bile and urine on recovery of 17-ketosteroids. In studying this problem, we have used a method involving the usual hydrolysis and continuous extraction. The Zimmermann reaction was then applied after removing the estrogens, or after separating the neutral 17-ketosteroid fraction with Girard's reagent, according to the general scheme outlined by Pincus (*Clinical Endocrinology* 5: 291). In either case, 2 color spectrophotometry was the final step. Additions of glucose to solutions of free 17-ketosteroids (androsterone and dehydroisoandrosterone), or to urine, did not influence the results. Addition of bile salts or purified human serum albumin to solutions of free 17-ketosteroids did not affect the results when solutions were analyzed without preliminary hydrolysis, but lowered them when hydrolysis was included. Low values due to addition of protein could be overcome by doubling the extraction period, but bile acids apparently increased the destruction of ketosteroids during hydrolysis, so that longer extraction did not influence the result. Analysis of urines with and without addition of bile salts or purified human serum albumin showed that the same findings apply when one deals with conjugated 17-ketosteroids. Effects of ultrafiltering to remove protein, and of varying the acid used in hydrolysis, were also studied.

Intravenous administration of commercial cytochrome C to rats previously treated with radioactive iron. HELMUT BEINERT and KURT R. REISSMANN (introduced by FRED W. OBERST). *USAF School of Aviation Medicine, Randolph Air Force Base, Texas.* The biosynthesis of iron-labeled cytochrome c showing a radioactivity suitable for tracer studies has been reported previously (*Science* 108: 634, 1948). Experiments were made to obtain knowledge about the penetration of injected cytochrome c into tissue cells. Two pairs of litter-mate rats previously treated with radioiron were nephrectomized unilaterally. One animal of each pair was then injected intravenously with commercial cytochrome c (8 mg.). Immediately thereafter, both pairs were taken to a simulated altitude of 20,000 ft. to favor utilization of injected cytochrome, if such actually does occur. All animals were sacrificed 24 hours after injection, to assure return of tissue cytochrome concentration to pre-injection level. Kidneys, hearts, and skeletal muscle were assayed for cytochrome concentration and the isolated cytochrome was checked for radioactivity. Comparative tests showed that the cytochrome content per unit weight of the left and right kidney removed under the conditions of the experiment generally does not differ by more than ±5%. Unilateral nephrectomy, therefore, represents a useful means of internal control. By this technique and comparison with the control litter-mates no significant elevation of the cytochrome values in the organs of the injected animals was found, indicating that no appreciable quantity of cytochrome was left in intercellular spaces. No significant dilution of the originally occurring radioactive cytochrome could be demonstrated in the cytochrome-injected animals. Iron analysis indicates that at least 94% of the measured radioactivity is due to cytochrome iron. This experiment does not give evidence of any significant incorporation of injected cytochrome c into tissue cells.

The fate of ingested uric acid in man. AARON BENDICH (by invitation), WILLIAM D. GEREN (by invitation), OSCAR BODANSKY and GEORGE BOSWORTH BROWN. *The Sloan-Kettering Institute for Cancer Research, New York, N. Y.* A normal male ingested 150 mg. of uric acid labeled with an excess of N^{15} in positions 1 and 3. Total urine collections were made over specific periods for 7 days and were analysed for total nitrogen, uric acid, urea and ammonia. For isotopic nitrogen analyses, uric acid was isolated by adsorption on Norite, elution with dilute alkali and purified by repeated crystallization. Urea was obtained as the dixanthydrol derivative and urinary ammonia was isolated. The course of the urinary excretion of each of the stated compounds, as well as their isotope content, was determined. During a period of 4 days, 80% of the administered isotope appeared in the urine. Of this amount, about ½ was present as urea N and about ⅓ as uric acid N. The results are discussed with respect to the question of uricase in human tissues, to bacterial action in the intestine, and to the relative contribution of purine nitrogen to urinary urea and uric acid nitrogen.

Human uric acid metabolism. JEAN D. BENEDICT (by invitation), PETER H. FORSHAM (by invitation) and DEWITT STETTEN, JR. *Dept. of Biological Chemistry, Harvard Medical School, Dept. of Medicine, Peter Bent Brigham Hospital, Boston, Mass.; and the Div. of Nutrition and Physiology, Public Health Research Institute of The City of New York, New York City.* Uric acid has been synthesized in such a fashion as to include a high concentration of N^{15} in the pyrimidine ring and this material has been administered intravenously in tracer dose to human subjects while maintained in balance on a purine-poor diet. From the concentrations of N^{15} in the urinary uric acid samples in the succeeding period it has been possible to estimate: *1*) the quan-

tity of uric acid which mixed with and diluted the injected isotopic uric acid; 2) the rate of *in vivo* formation of uric acid in man under these circumstances. These quantities have been compared with the prevailing level of plasma uric acid during the experiment and with the independently determined rate of urinary uric acid excretion. Experiments have been conducted on both normal subjects and patients suffering from gout.

Partial purification and properties of hydantoinase. FREDERICK BERNHEIM, MARY L. C. BERNHEIM (by invitation) and G. S. EADIE (by invitation). *Depts. of Physiology, Pharmacology and Biochemistry, Duke University Medical School, Durham, N. C.* The enzyme in rat liver which hydrolyzes hydantoin to hydantoic acid has been partially purified by removing proteins insoluble in slightly alkaline and acid solutions and by dialysis. The enzyme remains in a clear solution 0.25 ml. of which hydrolyzes under optimal conditions 4.0 mg. of hydantoin in 15 minutes. The solution also contains an active esterase for simple esters but only traces of choline, demerol and procaine esterases. If the activity is measured by the liberation of CO_2 the end point depends on the *p*H probably because of the simultaneous formation of an NH_2 group. That the end point at any *p*H corresponds to the quantitative formation of hydantoic acid was proved by the determination of the latter with the diacetyl monoxine reagent. The enzyme does not hydrolyze parabanic acid, dimethyl- or methyl ethyl hydantoin but they inhibit its action on hydantoin. Its *p*H optimum is about 8.6. The reaction is monomolecular throughout the greater part of the hydrolysis. The relation between initial velocity and substrate concentration is of the Michaelis-Menten type. Preliminary experiments indicate a dissociation constant not far from 0.01, showing relatively small affinity between enzyme and substrate.

Conversion of carotene to vitamin A by the rat. J. G. BIERI (introduced by M. O. SCHULTZE). *Div. of Agricultural Biochemistry, Univ. of Minnesota, Minneapolis, Minn.* Further evidence for the conversion of carotene to vitamin A by hypothyroid rats is presented from studies of the blood serum vitamin A content of hypothyroid rats given carotene. Two of four groups of rats on a vitamin A-deficient ration were made hypothyroid by feeding 0.2% thiouracil. When the animals showed weight plateaus, definite xerophthalmia and no measurable serum vitamin A, 200γ of B-carotene in Tween 80-water (1:1) was given weekly for 5 weeks orally or intramuscularly. Xerophthalmia disappeared. The hypothyroid rats gained weight for 15–19 days only; the others continued to gain. Vitamin A in blood serum determined by the method of Bessey before, and 12 hours after, the last administration of carotene showed the following.

Thyroid	Carotene	Serum Vitamin A, γ%	
		Before	After
Normal	Oral	7.2	13.7
Hypothyroid	Oral	8.3	21.6
Normal	Intramuscul.	7.0	10.2
Hypothyroid	Intramuscul.	5.3	26.3

Several laboratories have reported that the small intestine is involved in the conversion of carotene to vitamin A by the rat. Preliminary studies show that vitamin A-deficient rats given 400γ of B-carotene in Tween intramuscularly after surgical removal of the small intesting have in the blood serum, after 5 hours, appreciable amounts (equivalent to 12–50 γ% of vitamin A) of a fat soluble light-sensitive material absorbing at 328 mμ. Operated control rats given Tween show no appreciable change in serum fat-soluble components absorbing at 320–350 mμ.

Cystathionase. FRANCIS BINKLEY. *Laboratory for the Study of Hereditary and Metabolic Disorders, Univ. of Utah School of Medicine, Salt Lake City, Utah.* The enzyme responsible for the conversion of cystathionine to a compound reacting in the test of Sullivan for cysteine has been obtained in a condition of high purification from rat and pig liver. The purified enzyme was obtained in crystalline form and was dissociated, under acid conditions, into an inactive crystalline protein and an alcohol-soluble coenzyme. The inactive protein was reactivated by incubation with the coenzyme or a boiled extract of liver at values of *p*H near 8. Magnesium ions and inorganic phosphate were required for the activity of the enzyme but it was not possible to demonstrate an uptake of phosphate in the reaction. The rate of reaction was dependent upon the concentration of inorganic phosphate as well as upon the concentration of cystathionine or enzyme. The enzyme was active in the desulfhydration of cysteine; inorganic phosphate was required for the desulfhydration of cysteine. Cysteine was not a primary product of the action of the enzyme on cystathionine; the product responded to the Sullivan reaction but not to the reaction of Nakamura and Binkley. If, however, the reaction product were incubated with crude extracts of liver or kidney, cysteine was produced. Whereas iodometric titrations indicated a complete cleavage with the purified enzyme, the Sullivan reaction indicated less than 40% cleavage.

Hydrolysis of conjugated 17-ketosteroids in urine concentrates by acetate buffers. JOEL BITMAN (by invitation) and SAUL L. COHEN. *Dept. of Physiological Chemistry, Univ. of Minnesota Medical School, Minneapolis, Minn.* Butanol extracts of male urine prepared for studies on the hydrolysis of concentrates of the conjugated ketosteroids

showed a considerable loss (up to 45%) of these conjugates from the butanol extracts if the extraction was carried out at *p*H above 3.0. Similar losses occurred if the butanol concentrates were washed with buffers of *p*H above 3.0. This indicates the presence in urine of some conjugated ketosteroids whose salts are more soluble in water than in butanol. The $BaCl_2$-acetate buffer hydrolytic technique of Talbot *et al* (1943) when applied to butanol extracts of normal male urine liberated 20–40% of the total ketosteroids, of which 40–60% was of the beta type. These amounts were independent of the *p*H of the urine existent when the butanol extracts were prepared. The same degree of hydrolysis occurred even when the $BaCl_2$ was omitted from the hydrolytic mixture and the butanol residues were heated with buffer alone. Hydrolysis with *p*H 5.8 acetate buffer was found to be 85% complete in 4 hours. No marked increase in the proportion of ketosteroids liberated by buffers occurred when the buffer *p*H was reduced until a *p*H of 3.0 or less was achieved. These results tend to indicate the presence in normal male urine of a specific group of conjugates whose salts are much more soluble in butanol than in water and which are sufficiently labile to be hydrolyzed by relatively mild conditions.

Influence of magnesium and cobalt on the inhibition of phosphatases by α-amino acids. Oscar Bodansky. *Memorial Hospital for The treatment of Cancer and Allied Diseases and the Sloan-Kettering Institute for Cancer Research, New York City.* In the presence of inhibiting concentrations of α-amino acids or cyanide the usual activating effect of magnesium on bone phosphatase was decreased and in some instances was transformed into a retardant effect. The extent of the activating effect of cobalt on bone phosphatase was decreased slightly as the concentration of amino acid was increased. In the conjoint presence of cobalt and magnesium, accomplished either before or after the start of the reaction, the degree of activation of bone phosphatase which had been inhibited by amino acids was much greater than with either cobalt or magnesium alone and counteracted to varying degrees the inhibition by amino acid. The activating effect of magnesium on intestinal phosphatase was unaffected as the concentration of amino acid was increased; the activating effect of cobalt was decreased. The extent of activation of intestinal phosphatase which had been inhibited by amino acids was about the same in the conjoint presence of cobalt and magnesium as in the presence of magnesium alone. Human osteogenic sarcoma phosphatase resembled normal rat bone phosphatase with respect to the extent of inhibitions by various amino acids and the influence of cobalt and magnesium on these inhibitions. The present findings are interpreted to indicate that α-amino acids inhibit bone, intestinal and osteogenic sarcoma phosphatases by combining with an essential metal component of these enzymes and that this component normally mediates the activation of bone and osteogenic sarcoma phosphatases, but not of intestinal phosphatase, by magnesium.

Quantitative separation and determination of small amounts of histidine and tyrosine employing paper chromatography. Diana Bolling (by invitation), Herbert A. Sober (by invitation) and Richard J. Block. *Dept. of Biochemistry and Physiology, New York Medical College, Flower and Fifth Ave. Hospitals, New York City.* Pauly (1904) reported that histidine and tyrosine gave a red color when treated in alkaline solution with freshly diazotized sulfanilic acid. The use of this reaction for quantitative determinations has been limited by the difficulties in obtaining complete separation of these 2 amino acids. The excellent technique for the separation of amino acids by paper chromatography, introduced by Consden *et al* (*Biochem. J.* 36: 224, 1944), and the adaptation of this method for their estimation, based on the observation that the concentration of an amino acid on the paper was proportional to the area of the spot multiplied by the color density (Block, *Science* 108: 608, 1948), suggested that the Pauly reaction could be readily adapted to the determination of histidine, tyrosine, etc. Method: Histidine and tyrosine are separated by chromatography using a mixture of n-butanol, 100 parts, glacial acetic acid, 10 parts, saturated with water. Ascending chromatograms on S&S 598 or 596 paper are run for 3 hours. The paper is removed, dried, and sprayed with freshly diazotized sulfanilamide dissolved in n-butanol. The paper is allowed to air-dry for exactly 5 minutes. It is then sprayed with an emulsion of 5% sodium carbonate in 2 volumes of butanol. The chromatograph is air dried and the quantities of histidine and tyrosine are determined from the product of the color area times density. The results obtained on casein, zein and hemoglobin agree with the better values in the literature.

Influence of pituitary and adrenal glands on ketone body production by rat liver slices. Philip K. Bondy (by invitation) and Alfred E. Wilhelmi. *Dept. of Physiological Chemistry, Yale Medical School, New Haven, Conn.* Slices of rat liver were incubated in Krebs Ringer medium, with phosphate buffer, *p*H 7.4, in oxygen at 38°C. Ketone bodies were determined by the method of Greenberg and Lester on the total contents of the flask. All animals were fasted 18 hours before the experiment. Livers from normal rats produce 2.4 ± .16 μM/100 mg. (dry weight) in 15 minutes, and 8.8 ± .46 μM/100 mg. in 120 minutes, an increment of 3.5 ± .43 μM/100 mg/hour. The rate of appearance of ketone bodies was constant from 5 minutes to 120 minutes. Livers from adrenalectomized rats formed 2.5 ± .19 μM/100 mg. in 15 min-

utes, and 10.2 ± .43 μM. in 120 minutes, an increase of 4.4 ± .29 μM/100 mg/hour. These results are not significantly different from the data obtained from normal rat livers. The livers of rats hypophysectomized several months previously produced 1.5 ± .10 μM/100 mg. in 15 minutes, and 3.7 ± .20 μM/100 mg. in 120 minutes, a rate of 1.3 ± .20 μM/100 mg/hour. These values are significantly lower than those obtained with either normal or adrenalectomized animals. In normal rats, the body weight was found to be a significant factor, since liver slices from heavier rats produced more ketone bodies than did slices from lighter animals (r = 0.8428; p less than 0.05). This correlation was observed only in the 120-minute period. No such relationship between body weight and ketone body formation could be demonstrated for hypophysectomized or adrenalectomized animals.

A microbiological determination of D-phenylalanine. ERNEST BOREK and HEINRICH WAELSCH. *Depts. of Biochemistry, New York State Psychiatric Institute and Columbia Univ., New York City.* The microbiological determination of D-amino acids has been previously achieved by the use of two different varieties of microorganisms one of which utilizes only the L-amino acid, and the other, either the D or the D-L form. During studies of the metabolism of phenylalanine and its derivatives in *Lactobacillus Arabinosus* we found that, while these organisms can utilize only L-phenylalanine, they readily metabolize both optical antipodes of phenyllactic acid in lieu of the amino acid. Since the growth response of the microorganisms is nil to D-phenylalanine but complete to D-phenyllactic acid, and, since the chemical transformation of the amino acid to its hydroxy analogue is almost quantitative, a convenient determination of the D-amino acid is possible. Samples of D-L phenylalanine in microgram quantities, produce, after reaction with nitrous acid, almost twice the growth response of an identical, untreated aliquot. The blood of patients with oligophrenia phenylpyruvica has been subjected to a search for D-phenylalanine which has been claimed to accumulate in these subjects. Within the limits of error of the method the phenylalanine, which circulates in concentrations as high as 30 mg.% the blood of these subjects is the L-isomer.

Incorporation of labeled lysine into the proteins of guinea pig liver homogenate. HENRY BORSOOK, CLARA L. DEASY (by invitation), ARIE J. HAAGEN-SMIT (by invitation), GEOFFREY KEIGHLEY (by invitation) AND PETER H. LOWY (by invitation). *Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, Calif.* The incorporation of L-lysine (labeled with C^{14} in the ε-position) into the proteins of guinea pig liver homogenate was studied using either the whole homogenate or the sedimented fraction obtained by centrifuging the 15-fold diluted homogenate at 2500 g. Characteristics of the reaction with the whole homogenate are: its optimum *p*H is at 6.1; the presence of calcium is obligatory; the reaction proceeds as well in nitrogen as in oxygen; it is inhibited nearly completely by fluoride, and less completely by arsenate, arsenite, azide, cyanide and dinitrophenol; the concentration of L-lysine incorporated is approximately a linear function of the lysine concentration and is independent of the homogenate concentration. Characteristics of the reaction with the sedimented fraction are: its optimum *p*H is approximately 7.3; calcium accelerated the reaction only slightly and its presence is not obligatory; it proceeds under nitrogen but more slowly than under oxygen; it is inhibited slightly by fluoride and practically uninhibited by arsenate, arsenite, azide, or dinitrophenol; the concentration of lysine incorporated is a function of the total amount of lysine in the reaction mixture. The rate of incorporation of lysine into the proteins of the whole homogenate is approximately the same as that *in vivo*; the rate with the sedimented fraction is several times faster.

Passage of hemoglobin through the glomerular membranes and its excretion by the amphibian kidney. PHYLLIS A. BOTT. *Dept. of Physiological Chemistry, Woman's Medical College of Pennsylvania, Philadelphia, Penna.* Kidneys of amphibia were perfused with Ringer's solution containing human hemoglobin prepared by Drabkin's method (*J. Biol. Chem.* 164: 703, 1946). 'Direct' glomerular puncture experiments and 'indirect' (hemoglobin-inulin) experiments were made with methods similar to those used previously with other proteins (Bott and Richards, *J. Biol. Chem.* 141: 291, 1941). Indirect experiments: Hemoglobin excretion in ureteral urine was influenced by *p*H as in the experiments of Webster, Engel, Laug and Amberson (*J. Cell. and Comp. Physiol.* 5: 399, 1934) with laked cells, and the percentage filtration calculated from these by the indirect method also seemed to be so influenced. In a number of cases the calculated percentage filtration of hemoglobin did not show an increase when the concentration of hemoglobin in the perfusion fluid was increased. Direct experiments: From glomerular collection analysis there appears to be considerable variation in the degree of completeness of hemoglobin filtration by the individual glomeruli, but possibly a trend toward more complete filtration with increasing concentration of hemoglobin in the perfusion fluid. Glomerular fluid and ureteral urine were collected simultaneously in a number of experiments.

Antithromboplastic activity of navy bean trypsin inhibitor in vivo. DONALD E. BOWMAN. *Dept. of Biochemistry and Pharmacology, Indiana Univ.*

School of Medicine, Indianapolis, Ind. A highly active and abundant navy bean trypsin inhibitor has been used to retard the *in vivo* coagulation which results from the intravenous injection of trypsin in rabbits. This inhibitor was prepared by a modification of an earlier procedure employing isoelectric precipitation of inert proteins, fractional salting out of the active material and fractionation with alcohol. In rabbits the navy bean trypsin inhibitor confers a considerable degree of protection against the lethal effects of single large doses of intravenously injected trypsin or repeated small doses of the enzyme. Extensive pulmonary embolism which is almost invariably observed upon immediately examining rabbits killed with trypsin alone is not found upon sacrificing those receiving the enzyme plus its inhibitor. In rabbits receiving repeated small injections of trypsin the coagulation time of carotid blood is first observed to increase markedly and then gradually decrease, finally reaching a point considerably below the control level at the time of fatal termination. The initial rise is also observed with the enzyme plus its inhibitor; however, the subsequent fall is not as severe. Iodinated trypsin which retains relatively more of the proteolytic activity as compared with the hypotensive effect of the original enzyme also gives rise to pulmonary emboli particularly with repeated small doses. Single large doses have been less toxic than the untreated enzyme given in amounts sufficient to provide equivalent proteolytic capacity. Evidence of anaphylactic reactions was not observed upon intravenous injection of the inhibitor at 14-day intervals.

Tuberculostatic properties of autoclaved human plasma. DONALD E. BOWMAN. *Dept. of Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis, Ind.* During an investigation of enzymatic factors in tuberculosis it has been observed that undiluted autoclaved human plasma containing glycerol is less able to support growth of the H37 strain of tubercle bacilli than is sterile plasma which has been coagulated at 80° for 10 min. On the latter surface growth of the organisms having typical staining properties can be observed in 2 to 4 days after inoculation while growth on plasma which has been autoclaved for 15 min. at 15 lb. pressure requires a week or more to reach a comparable point and subsequently shows much less growth. With non-autoclaved dog plasma or restored dessicated human plasma growth is comparable to that on autoclaved fresh human plasma. That the addition of a concentrate of the fluid derived from autoclaved human plasma to synthetic media consistently inhibits growth is readily observed and can be estimated by determining the nitrogen content of the washed organisms by the procedure described by Youmans. Fluid derived from plasma coagulated at 80° does not inhibit growth. In fact in less favorable media such as that in which the yeast extract and surface active accelerating agents have been omitted from Dubos' synthetic media, fluid pressed from 80° coagulated plasma actually accelerates growth. Uric acid shows an inhibiting effect which it partially loses upon being autoclaved.

A biologically-active, reversible oxidation product of α-tocopherol. PAUL D. BOYER. *Div. of Biochemistry, Univ. of Minnesota, St. Paul, Minn.* A biologically-active oxidation product of α-tocopherol has been formed by reaction with two equivalents of Fe^{+++}/M and isolated as an unstable, colorless oil by extraction and chromatographic procedures. It has an absorption maximum at 237 mμ. in isooctane ($\epsilon_M = 1.2 \times 10^4$), and is readily reduced to tocopherol by ascorbic acid or hydrosulfite. The product is a structural isomer of α-tocopherylquinone, $C_{29}H_{50}O_3$, into which it is readily converted by a slight excess of Fe^{+++} and by acids and alkalies. Its antisterility activity is 1/10 parenterally and 1/30 orally of that of α-tocopherol. This suggests a relation of the oxidation product to the biochemical function of tocopherol. Tentatively the product is considered to be 2,5,7,8-tetramethyl-2-(4′,8′,12′-trimethyltridecyl)-6-keto-7-hydro-8,9-epoxychroman. The absence of an OH group is demonstrated by failure to react with reagents for OH groups and by the infra red absorption spectrum. The hetero ring is thus intact and the extra oxygen must be associated with the carbon ring in a cyclic or peroxide structure. The product liberates I_2 from NaI in Ac_2O, and this property, together with the ease of conversion to the quinone, has prevented direct reaction with carbonyl reagents. The ultraviolet absorption spectra and other properties of the molecule are best explained by an α-β unsaturated keto structure. Similar products from γ- and δ-tocopherols show maxima about 10 mμ. shorter wave length, consonant with the postulated structure. Properties of the molecule and structural considerations suggest the location of the extra oxygen in a three-membered ring in the 8-9 position.

Evaluation of some lactic acid producing bacteria in the microbiological assay of amino acids. MYRON BRIN (by invitation) and HAROLD H. WILLIAMS. *Dept. of Biochemistry and Nutrition and The School of Nutrition, Cornell Univ., Ithaca, N.Y.* The amino acid assay of complex feedstuffs containing relatively large amounts of lipids, complex carbohydrates, or both, offers difficulties not apparent in the assay of purified protein materials. This report deals with the comparative analysis of complex feeds by 5 bacteria previously proposed for amino acid assay. Whole egg, soybean oil meal, and clover hay were chosen as representative of

feedstuffs, and casein was included as a standard of comparison with literature values. Depending upon the amino acid requirements for the respective organisms, comparative assays for the essential amino acids were run on the above materials with *Lactobacillus arabinosus* 17-5, *Lactobacillus casei*, *Lactobacillus delbruckii* LD5, *Leuconostoc mesenteroides* P-60, and *Streptococcus faecalis*. In order to measure differences of response between organisms to the test materials, a uniform basal medium was developed which is applicable to the 5 bacteria used. Comparative results indicate that all of the organisms do not yield equivalent amino acid values under similar conditions. It is concluded that under the conditions of this study 3 bacteria yielded the most accurate values, namely: *Streptococcus faecalis*—arginine, histidine, lysine, methionine, threonine, tryptophan and valine; *Leuconostoc mesenteroides* P-60—histidine, lysine, methionine and phenylalanine; *Lactobacillus arabinosus* 17-5—isoleucine, leucine, and tryptophan.

Mechanism of histidine synthesis in lactic acid bacteria. HARRY P. BROQUIST (by invitation) and ESMOND E. SNELL. *Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wis.* If *Lactobacillus arabinosus* 17-5 is cultured in a medium deficient in histidine and purine bases, the organism grows very slowly. Much more rapid growth is obtained on the addition of either histidine or individual purine bases. This suggests a relationship between histidine and purine bases in metabolism. The possibilities (reaction *a*) that histidine may serve as a precursor of purines, or (reaction *b*) that the purines may serve as a precursor of histidine are considered. Reaction *a* has been frequently proposed, and appears unlikely in view of present knowledge. Furthermore, the histidine requirement of *Streptococcus faecalis* R is not increased by culturing in the absence of the proposed product, purine bases. Data to be presented indicate rather that the purine bases serve as a precursor for the imidazole ring of histidine (reaction *b*) and that xanthine is particularly effective in this relationship. In the absence of vitamin B_6, histidine becomes essential for growth of *L. arabinosus* and cannot be replaced by purine bases, implying that vitamin B_6 is involved somewhere in reaction *b*. Imidazole pyruvic acid replaces histidine for both *L. arabinosus* and *S. faecalis*, but only when vitamin B_6 is present in the medium. Thus B_6 appears to function in conversion of this keto acid to the amino acid. If imidazole pyruvic acid is the immediate precursor of histidine this implies a wider significance for the transamination reaction than has recently been indicated.

Changes in sodium sulfate solutions in the upper and lower intestines of dogs. GLADYS R. BUCHER (by invitation), CARL E. ANDERSON (by invitation) and C. S. ROBINSON. *Dept. of Biochemistry, Vanderbilt University, Nashville, Tenn.* The ability of the small intestine to alter sodium sulfate has been studied in a double period test in a series of acute dog experiments using 2 methods of isolating the gut segments, and also in chronic dogs with Thiry Villa loops. In the acute experiments, segments were isolated using the terminal end of the Miller-Abbott apparatus in the jejunum and 60 cm. from the ileocecal valve, or by mechanical obstruction with flat clamps in these regions. After a sulfate wash, each loop was filled with Na_2SO_4 (35–40 cc., 204 mEq/l.) and drained for analysis after 20 minutes. Residual volume was obtained by determining the conductivity of an equal volume of 10% sucrose solution used as a rinse. Following a rinse with sulfate, a second 20-minute test was made. The analysis consisted of determining volume, *p*H, total base, ammonia, sulfate, chloride and total CO_2. The data from the 3 types of preparation were statistically homogenous. The first period samples in both upper and lower loops showed some significant differences from the second period samples. In upper loops, the second period samples were significantly different in possessing a more acid *p*H, higher sulfate and a lower chloride concentration. In the lower loops, the second period samples were significantly different in possessing a more acid *p*H, increase in total base concentration and a decrease in the concentration of ammonia. These ionic shifts occurred when no consistent differences in volume were detected. The sample differences bespeak a reduction in the rate of ionic shifts in the second period, due possibly to a fatigue or saturation phenomenon.

Anaerobic and aerobic glycolysis of parasitic tissue helminths. ERNEST BUEDING. *Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* In contrast to tissues from vertebrates and to many bacteria, lactic acid is not the major end product of the anaerobic carbohydrate metabolism of parasitic helminths living in the intestinal tract or the bile ducts. However, lactic acid production accounted for at least 80% of the total amount of carbohydrate utilized anaerobically by 2 tissue parasites, the filarial nematode, *Litomosoides carinii*, and the trematode, *Schistosoma mansoni*. This was established by isolation and identification of the benzimidazole and the p-bromphenacyl derivatives from media in which the parasites had been incubated in the presence of glucose. Glycolysis of *Litomosoides carinii* was greatly reduced by aerobiosis and inhibition of its respiration resulted in a compensatory increase of glycolysis. In schistosomes and in another tissue parasite, the filarial nematode, *Dracunculus insignis*, the rates of glucose utilization and of lactic acid production remained

constant under anaerobic and aerobic (1–100% oxygen) conditions, and were not affected by inhibitors of respiration. Evidence will be discussed indicating that a large amount of energy derived from oxidative reactions is essential for survival of *Litomosoides carinii*, but not for that of schistosomes, in which respiration and glycolysis occur independently of each other. Glycolysis of the parasites was reduced markedly by low concentrations of —SH inhibitors. This effect could not be reversed by glutathione, dithiopropanol or H_2S. Dithiopropanol (but not glutathione) greatly enhanced the inhibitory action of p-chloromercuric benzoate on glycolysis of the schistosomes.

Bacterial glucuronidase. HENRY J. BUEHLER (by invitation), PHILIP A. KATZMAN and EDWARD A. DOISY. *Laboratory of Biological Chemistry, Saint Louis Univ. School of Medicine, Saint Louis, Mo.* The estimation of several important substances excreted in the urine as glucuronides depends upon as initial hydrolysis. Since the prevailing methods for hydrolyzing such substances result in considerable destruction, we have directed our efforts toward the preparation of potent glucuronidase extracts of bacterial origin. Of fourteen organisms grown in a 1% peptone medium only *C. zerose*, *C. hoffmani* and *E. coli* produced glucuronidase as indicated by the hydrolysis of phenolphthalein glucuronide. *E. coli* was selected for further study because it showed the greatest glucuronidase production under these conditions. The optimum *p*H for enzyme production by this organism at 25°C. in a peptone-beef extract medium containing 0.05M phosphate buffer was found to be about 7.3. The optimum *p*H for enzyme activity using phenolphthalein glucuronide as the substrate and 0.033M KH_2PO_4 as buffer was determined to be approximately 6.2. The production of glucuronidase by *E. coli* can be markedly increased by the addition of menthol glucuronide to the growth medium. The addition of 0.5% menthol glucuronide in this manner has effected as high as a 70-fold increase in the yield of the enzyme. Employing as the enzyme preparation the culture fluid after dialysis and filtration, phenolphthalein glucuronide was quantitatively hydrolyzed in 2 hours. The extent of hydrolysis for shorter periods was not measured.

***l*-Alanine oxidase of rabbit kidney and liver.** M. V. BUELL and J. L. STILL (introduced by D. E. GREEN). *Institute for Enzyme Research, Univ. of Wisconsin, Madison, Wis.* Liver and kidney cyclophorase preparations can catalyze the complete oxidation of *l*-alanine to CO_2, NH_3 and H_2O. This process requires the presence of some member of the citric acid cycle in sparking concentration. It appears that the first step in the complete oxidation is a transamination between alanine and α-ketoglutarate with formation of pyruvate and glutamate. Once the process is properly started the oxidation of pyruvate and glutamate both of which pass through α-ketoglutarate as an intermediary step provide a continuous supply of α-ketoglutarate for the initial transamination reaction. The alanine-glutamic transaminase is capable of working at catalytic concentrations of α-ketoglutarate. Under appropriate conditions the cyclophorase system can synthesize *l*-alanine from pyruvic acid and NH_3. This synthesis involves both the synthesis of glutamate from α-ketoglutarate and NH_3 and the reversal of the transamination reaction: 2 α-ketoglutarate + NH_3 → glutamate + succinate glutamate + pyruvate → α-ketoglutarate + alanine. The synthesis of glutamate from α-ketoglutarate and NH_3 proceeds very rapidly under aerobic conditions. α-Ketoglutarate in excess suppresses almost completely ammonia production from either glutamate or alanine in accordance with the dictates of the above equations.

Effect of amino acid administration on amino acid oxidase activity. R. N. CAGAN (by invitation), J. L. GRAY (by invitation) and H. JENSEN. *Medical Dept. Field Research Laboratory, Fort Knox, Ky.* The effect of intraperitoneal injection of an amino acid mixture on the amino acid oxidase activity of the liver and kidney was studied in normal, hypophysectomized, adrenalectomized and thyroidectomized rats. The enzyme activity of a buffered extract of homogenized tissue was measured. Simultaneous determinations of blood amino acids, urea and glucose were made. An increase in the amino acid oxidase activity of the liver of normal animals was found after amino acid administration while no such increase was observed in the operated animals. The amino acid oxidase activity of the kidney was found to be dependent principally upon the amino acid level of the blood and not upon hormonal control. Comparison of the amino acid oxidase activity of normal and operated animals was made.

Direct utilization of sucrose by Leuconostoc. WARNER W. CARLSON and VIRGINIA WHITESIDE CARLSON (introduced by EMMETT B. CARMICHAEL). *Biochemistry Dept., Medical College of Alabama, Birmingham, Ala.* Several instances of direct utilization of disaccharides by microorganisms have been reported in the literature. With the disaccharide as the carbohydrate source, earlier growth and acid production were observed than resulted from the use in the media of the constituent monosaccharides. In the case of *Leuconostoc mesenteroides* and *L. dextranicum*, direct utilization of sucrose has been indicated as a result of the fact that these organisms synthesize dextran only from the intact disaccharide, no polysaccharide being formed when glucose, fructose, or a mixture of the monosaccharides are employed. We have

observed earlier growth and more rapid acid production from sucrose by various strains of *Leuconostoc* regardless of whether a particular strain formed large amounts or only a trace of the polysaccharide. Investigation of the growth requirement of certain strains of *Leuconostoc* in a chemically defined sucrose medium, and in media containing the constituent monosaccharides revealed several differences, the most important of which was an apparent absence of a biotin requirement when the disaccharide was employed, as compared with a need for this vitamin when glucose or fructose were employed. Since sucrose often is known to contain significant amounts of biotin, the studies were also made with media containing different amounts of raw egg white.

Toxicity studies with demerol hydrochloride (isonipecaine) on adult male guinea pigs. EMMETT B. CARMICHAEL, WALTER H. JOHNSON (by invitation) and FRANK A. KAY (by invitation). *Biochemistry Dept., Medical College of Alabama, Birmingham, Ala.* Toxicity studies have been made with Demerol hydrochloride on adult male guinea pigs. A fresh aqueous solution was injected intraperitoneally. The doses varied by 10 mg. increments from 80 to 170 mg/kg. The smallest dose to cause death was 110 mg/kg. while a dose of 170 mg/kg. killed 100% of the animals. With the large doses, animals usually became nervous and excitable within 5 to 10 minutes and as the symptoms became more severe, convulsions developed. Then an animal would fall on its side during a convulsion and if the dose was lethal, the animal usually would not stand up before expiring. The survival time for those animals that died varied from 12 to 323 minutes with a majority of the animals dying in less than 2 hours. The LD_{50} of Demerol for adult male guinea pigs seems to be in the range 130 to 150 mg/kg.

Separation of three naturally occurring adenine ribonucleotides by paper chromatography and ion-exchange. C. E. CARTER (by invitation) and WALDO E. COHN. *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.* Adenylic acid prepared from yeast nucleic acid was assigned the structure of adenosine-3-phosphoric acid by Levene. It has been found by techniques of paper chromatography that yeast adenylic acid may be resolved into two components and by an anion exchange procedure these two components may be separated from each other and from muscle adenylic acid. The two adenylic acids in yeast nucleic acid are degraded to adenine by acid hydrolysis and to adenosine by weak alkaline hydrolysis and kidney nucleotidase. Neither component is desaminated by Schmidt's muscle adenylic acid desaminase and the phosphorous of both is acid labile. These findings suggest that nucleic acid contains adenosine-2-phosphoric acid and adenosine-3-phosphoric acid (the 1 and 4 positions on the ribose carbon chain being excluded by the furanose ring and the 5 position by the acid labile nature of the phosphorous of yeast adenylic acid) although other possibilities for isomerism are not excluded. The ion-exchange separation of adenylic acids was achieved by elution with dilute formic, acetic or hydrochloric acid solutions from strong-base anion exchangers. Separation of nucleotides by paper chromatography was obtained in a two phase system consisting of isoamyl alcohol and 5% Na_2HPO_4 in water.

Inositol lipids of soybean. HERBERT E. CARTER, WALTER D. CELMER (by invitation), MACK H. MCCORMICK (by invitation), CLIFFORD O. NYMAN (by invitation) and FRANCES W. SAUNDERS (by invitation). *Div. of Biochemistry, Noyes Laboratory of Chemistry, Urbana, Ill.* Attempts to prepare inositol lipid from soybean by the procedure of Woolley (*J. Biol. Chem.*, 147: 581, 1943) yielded non-homogeneous products containing a non-reducing carbohydrate which gave positive Seliwanoff and diazouracil tests. Therefore, other fractionation procedures were studied, using crude-soybean phosphatide from several commercial sources. The crude material was freed of oil and the product (3–4% inositol) was extracted with glacial acetic acid. The residue was a crude inositol lipid (10–13% inositol) which contained about 80% of the original inositol. Addition of hexane to the glacial acetic acid extract gave a white precipitate (2–5% inositol) containing a large amount of the non-reducing carbohydrate. This material when freed of inositol gave a positive test for galactose and sucrose. The optical rotation and the properties of the acetyl derivative were similar to those of raffinose. The acetic acid insoluble fraction was further purified by distribution between aqueous acetic acid and cyclohexane giving a white powder containing 11–14% of inositol (Seliwanoff test negative). Distribution of this material between hexane and methanol gave two inositol-containing fractions.

Variability in the Beckman spectrophotometer. W. O. CASTER (by invitation) and OLAF MICKELSEN. *Nutrition Section, U. S. Public Health Service, Bethesda, Md.* Different Beckman Spectrophotometers may yield $E^{1\%}_{1\,cm}$ values differing by as much as 1–5% (Ewing and Parsons, *Anal. Chem.* 20: 423, 1948). One instrument was subjected to a series of control studies in order to determine the cause of this variation. Analysis of variance revealed several distinct and statistically significant sources of variability. Under constant conditions duplicate readings can be repeated within σ = 0.2%. This is only slightly above the error in reading the instrument scale. This has often been considered the 'true error' of this instrument. Quartz cell correction factors, (i.e. to compensate

for the lack of agreement between readings obtained from different cells when all are filled with identical solutions) change with changes in wave length (and simultaneous changes in slit width) to give an additional variation of 0.2%. From the circuit the phototube appeared to be by far the greatest source of inconsistency. The variation between a series of ultraviolet-sensitive phototubes was $\sigma = 1.1\%$ and between red-sensitive and ultraviolet-sensitive phototubes in their overlapping range, 600–625 mμ., was $\sigma = 3.2\%$. Changes in slit width over an 0.08 mm range resulted in a 0.9% variation in $E^{1\%}_{1\,cm}$ values. Changes in wave length over a 23 mμ. range likewise resulted in a 0.5% variation. Together these error factors are considerably greater than the 0.2% so often reported as the variability of this instrument. In the exact interpretation of $E^{1\%}_{1\,cm}$ values these factors should be considered.

Fractionation and chemical studies of proteins of rat liver. ALFRED CHANUTIN, E. C. GJESSING and STEPHAN LUDEWIG. *Biochemical Laboratory, Univ. of Virginia, Charlottesville, Va.* The proteins of saline extracts of rat liver have been fractionated by isoelectric precipitation and ethanol. A relatively large fraction containing a protein-lipide-nucleic acid complex was separated by isoelectric precipitation. A series of proteins were obtained by adjusting the ethanol and hydrogen-ion concentrations. These fractions were analyzed for their nitrogen, lipide and nucleic acid contents. Data for the distribution of arginase, phosphatase, catalase, esterase, succinic dehydrogenase and fluorophosphatase in these fractions will be presented.

Concentration of amino acids by the rat diaphragm suspended in artificial media. HALVOR N. CHRISTENSEN. *Children's Hospital and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* A study has been made of the activity of excised rat diaphragm in maintaining cellular amino acids at much higher concentrations than those of the extracellular fluid. Two procedures were used: *1)* One hemidiaphragm was analyzed immediately, the other after shaking in a given medium. The amino acid concentration of each was referred to the appropriate extracellular fluid; *2)* The two hemidiaphragms were shaken simultaneously in media identical except for one component. A solution containing 103 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 20 mM sodium pyruvate in an O_2-CO_2 atmosphere maintained the concentrating activity for glycine unchanged for 3 hours. α-Ketoglutarate served in place of pyruvate. When supplemented with pyruvate, rat plasma was equally satisfactory. The diaphragm glycine was responsive to the fluid glycine, falling at one fluid level and rising at a slightly higher one. In the absence of oxygen, or the presence of cyanide, arsenite, 80 mM potassium, or 2,4-dinitrophenol at 10^{-3} to 5×10^{-6} M, glycine was rapidly lost from the diaphragm. Pyruvate and dinitrophenol added to rat plasma stimulated and inhibited respectively the concentration of α-amino acids (measured collectively by ninhydrin). These observations relate to the energy source for amino acid concentration. All α-amino acids added to the medium at 21 mM concentration diminished by about ⅓ the glycine retained by the suspended diaphragm. Only α-amino acids had this effect, and pairs of optical antipodes were equally inhibitory. This inhibition, earlier shown in the intact animal, is interpreted as a competitive phenomenon.

Nitrogenous metabolism of the earthworm (Lumbricus terrestris). STANLEY COHEN (by invitation) and HOWARD B. LEWIS: *Dept. of Biological Chemistry, Medical School, Univ. of Michigan, Ann Arbor, Mich.* In the normal earthworm, the chief nitrogenous component of the excreta was ammonia (66–91%), with traces only of urea (<10%). During inanition, the urea content was greatly increased so that urea was the most important nitrogenous constituent of the excreta. Thus, after 18 days' fasting, from 82 to 86% of the nitrogen was present as urea and less than 10% as ammonia. When arginine was fed by tube to normal earthworms, the excretion of urea was greatly increased. Small increases in the amount of urea excreted were noted after the oral administration of citrulline, but not after ingestion of ornithine, glutamic acid, glutamine, glycine, alanine, histidine, or ammonium chloride. When citrulline was fed simultaneously with glycine, alanine, or glutamic acid, the urea excretion exceeded that observed when citrulline or the other amino acids were fed alone. The administration of ornithine with other amino acids did not alter the excretion of urea.

Detection of 7-keto-cholesterol in blood plasma. H. B. COLLIER and R. H. COX (by invitation). *Dept. of Biochemistry and College of Pharmacy, University of Saskatchewan, Saskatoon, Sask., Canada.* In connection with the administration of pure 7-keto-cholesterol to rabbits a method was sought for its detection in plasma filtrates. The Liebermann-Burchard reaction is negative; but with the Tschugaeff reagent (Trappe, *Z, Physiol. Chem.* 273: 177, 1942) the compound gives an intense yellow color with an absorption maximum at 432 mμ (Coleman model 11 spectrophotometer) and a green fluorescence. Cholesterol gives a pink color, with absorption maximum at 505 mμ, and a yellowish fluorescence. (A similar color, with maxima at 515–520 mμ, is also given by: cholestadiene (3:4, 5:6); coprostene-(4:5)-one-3; and dicholesteryl ether.) When the reaction is applied to dried acetone-alcohol filtrates of plasma, 7-keto-cholesterol may be detected by a peak in the spectrophotometric absorption curve at 432 mμ. However, with mixtures of cholesterol and 7-keto-cholesterol, nei-

ther the optical densities in the visible spectrum nor the fluorescence intensities are additive. Semiquantitative separation and estimation of cholesterol and 7-keto-cholesterol may be achieved by adsorption on alumina from petroleum ether, elution of cholesterol by chloroform and of 7-keto-cholesterol by acetone, followed by color development.

Demonstration of TPN dehydrogenases in the tissues of higher plants. ERIC E. CONN (by invitation), BIRGIT VENNESLAND and E. A. EVANS, JR. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.* Ammonium sulfate fractions of seven different higher plants which contain oxaloacetic carboxylase activated by manganous ions have been shown to contain a malic dehydrogenase requiring triphosphopyridine nucleotide (TPN) as a coenzyme. The overall reaction catalyzed by these preparations may be written

$$\text{malate} + \text{TPN}_{\text{oxidized}} \rightleftarrows \text{pyruvate} + \text{CO}_2 + \text{TPN}_{\text{reduced}}$$

and is analogous to the reaction catalyzed by the 'malic enzyme' of pigeon liver as described by Ochoa. The main difficulty in demonstrating the occurrence of the reaction in plant preparations is associated with the widespread distribution of enzymes which destroy TPN. This destruction can be prevented by muscle adenylic acid, adenosine triphosphate, diphosphopyridine nucleotide, and to a lesser extent, yeast adenylic acid, but not by nicotinamide or adenosine. The destruction of reduced TPN is accompanied by the disappearance of the absorption band at 340 mμ and therefore simulates a reoxidation of the nucleotide. The practical significance of these facts in connection with the procedures used to demonstrate the reaction will be discussed. A detailed study of the dehydrogenase from wheat germ has been made. Observations with parsley root, parsnips, beets, carrots, spinach and peas indicate a general similarity in the properties of the dehydrogenases and of the factors which destroy TPN in crude protein preparations from all these different plant sources.

Formation of acetoacetic acid from radioactive leucine. MINOR J. COON (by invitation), SAMUEL GURIN and D. WRIGHT WILSON. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* A fasted phlorhizinized rat was injected subcutaneously with a solution of 100 mg. of *DL*-leucine labeled in the β position with C^{14}. The acetoacetate which appeared in the urine was degraded by standard procedures. The acetone fraction was found to be radioactive, and iodoform prepared from it accounted for all of the isotope present. These findings are in accord with the view that the carboxyl group of leucine is lost early in the course of metabolism. In other experiments, the radioactive amino acid was incubated with rat liver slices. The acetoacetate formed was oxidized with permanganate to produce formic and acetic acids, both of which proved to be radioactive. It was calculated that the methyl and methylene carbons of the acetoacetate had approximately the same concentration of isotope. Accordingly, at some stage in the metabolism of leucine, its α and β carbons must split off as a two-carbon intermediate which is capable of condensing to form acetoacetate.

Effects of feeding an ethanolamine analogue (diethanolamine) on the formation of liver phospholipides. W. E. CORNATZER (by invitation), CAMILLO ARTOM and MARIETTA CROWDER (by invitation). *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.* With the aid of radioactive P as an indicator it was previously shown that the administration of a single large dose of diethanolamine to rats on low protein diets stimulates the lipide phosphorylation in the liver to a degree similar to, or greater than that observed after ethanolamine was given under the same conditions. With either substance, the stimulation involves both lecithin and cephalin fractions. The results are quite different when diethanolamine is added to the diets and fed over a period of 7 to 12 days. Under this condition the rate of lipide phosphorylation in the liver becomes markedly lower than in the controls on the same diets, unsupplemented or supplemented with ethanolamine. The decrease is mainly due to a diminished rate of formation of lecithins. Changes in the amount and distribution of liver phospholipides (increases in the total and non-choline containing phospholipides with a considerable decrease in the ratio lecithins to total phospholipides) were also noted. Indirect evidence was obtained for the presence of diethanolamine in the liver phospholipides of rats on diets supplemented with this compound. It is suggested that in these animals considerable amounts of diethanolamine-containing phospholipides are formed, and that these atypical phospholipides, being less easily metabolized than the natural cephalins, accumulate in the liver. This effect, in turn, would lead to a marked decrease in the formation of lecithins through a mechanism which remains obscure.

Electrophoretic patterns of plasma proteins during pregnancy and following delivery. MARGARET N. CORYELL (by invitation), ELIOT F. BEACH, and ABNER ROBINSON (by invitation). *Research Laboratory, Children's Fund of Michigan, Detroit, Mich.* The changes which occur in the plasma proteins of women at various intervals in pregnancy and postpartum are demonstrated by data obtained by use of the Tiselius electrophoresis technique with 89 plasma samples from the blood of 11 nonpregnant women, 23 women during pregnancy and postpartum, and the cords of 12 infants. Determinations were made with 3, 7, and 17 samples ob-

tained during the first, second, and third trimesters of pregnancy respectively; 16 maternal samples obtained at delivery; 10 samples within 5 days, and 13 samples 5 or more weeks postpartum. The data reveal a smooth progression of changes in all plasma protein fractions during the 3 trimesters of pregnancy. Comparison of the averages for non-pregnant patterns and those at delivery shows decreases in the absolute levels of gamma globulin of from 28 to 35%, respectively, while alpha$_1$, alpha$_2$, and beta globulins, and fibrinogen, increased 29, 41, 36, and 29%, respectively. During the 5 days following delivery there was some further fall in albumin and gamma globulins and a rise in alpha$_1$ globulin. By 5 to 9-weeks postpartum all subjects had plasma protein patterns similar to those of non-pregnant women, with complete restoration of the albumin fraction although gamma globulin was still reduced and alpha$_1$ and beta globulins were slightly elevated. Effects of factors such as milk flow, lactation failure, or renewed menstruation, upon the plasma protein patterns were not apparent.

Oral ingestion of histidine and the glutamic acid blood level in rats. H. R. CROOKSHANK and BERNARD F. CLOWDUS (introduced by EMMETT B. CARMICHAEL). *Biochemistry Dept., Medical College of Alabama, Birmingham, Ala.* The course of histidine metabolism and the conversion of histidine to glutamic acid was studied in previously fasted rats by assaying the blood for histidine, total imidazoles, glutamic acid, and amino nitrogen both before and at intervals after feeding single doses of L-Histidine. The histidine, total imidazoles, and amino nitrogen reached maximal concentrations in 1 hour after feeding and returned to the fasting level in 6 to 10 hours. No significant change in the glutamic acid level was observed, either before or at any interval after ingestion of L-Histidine. The data seem to indicate either little conversion of histidine to glutamic acid or that the glutamic acid thus formed is produced too slowly to be detected by our method or is converted to another metabolite which retains the alpha-amino nitrogen.

Incorporation of P^{32} during the operation of the cyclophorase system. R. CROSS, W. ATCHLEY and H. ALBAUM (introduced by D. E. GREEN). *Enzyme Laboratory, College of Physicians and Surgeons, Columbia University, New York City.* When the cyclophorase system is allowed to carry out the oxidation of any member of the citric acid cycle, of proline, glutamate and β-hydroxybutyrate in presence of radioactive inorganic phosphate it is found that the enzyme gel after thorough washing with 0.9% KCl is radioactive and the radioactivity is from 5–10 times as high as that of the blank treated in the same way except without added substrate. Under optimum conditions the radioactivity/μ mol of total phosphate extractable from the gel by treatment with 5% trichloroacetic acid approaches the value for the radioactivity/μ mol of inorganic phosphate in the original digest. Arsenite and capryl alcohol abolish this incorporation of radioactive phosphate. Gramicidin and dinitrophenol only partially inhibit the incorporation though both together reduce the level of radioactivity to the level of the blank without substrate. Analysis of the radioactive gel has shown that either inorganic phosphate or one or more substances which estimate as inorganic phosphate are largely responsible for the radioactivity. The fraction corresponding to the adenosine pyrophosphate is also highly radioactive and it has been shown by enzymatic procedures that the radioactivity actually resided in the pyrophosphate moieties of ATP and ADP. Incorporation of P^{32} accompanies the oxidation of succinic acid in the cyclophorase system but not oxidation in the classical succinic oxidase system.

Influences of dietary protein: transmittal of growth factor. FRANK A. CSONKA and M. W. OLSEN (by invitation). *Bureau of Human Nutrition and Home Economics and Bureau of Animal Industry, Agricultural Research Adm., U. S. D. A., Washington, D. C.* Evidence has been presented previously that the cystine and methionine content of a hen's egg may be increased by feeding a high-protein casein diet (Csonka, Denton, and Ringel, *J. Biol. Chem.*, 169: 259, 1947). In the present work, two pens of chickens were used to furnish eggs for incubation; one group was fed a high-protein diet of which 20% was casein and the other group was fed a low-protein diet containing no casein. Eggs were incubated from both pens at the same time and the chicks were reared together to 4 weeks of age on the same growing mash ration which had no casein. The chicks from the hens fed the casein diet grew faster than those from the hens fed the low protein diet, as shown by their greater body weight at 4 weeks of age. This difference in chick growth is related to the maternal dietary protein as the chief variable component in our experimental arrangement, the growth effect being transmitted through the egg to the chick. When the casein was replaced in the hen's ration with an equivalent quantity of soybean meal, the same accelerated growth effect was not observed. Addition of dried cow manure or Wilson's liver fraction 'L' to the chick mash inhibited the growth of the chicks that originated from the hens fed the high protein casein ration. The same supplements, however, slightly accelerated the growth of the chicks that had descended from parents fed the low protein ration. The growth of the chicks on the supplemented diet whose parents received a high protein soybean ration was not affected.

An ultraviolet spectrophotometric method for the determination of benzene hexachloride. BERNARD DAVIDOW and GEOFFREY WOODARD (introduced by R. L. GRANT). *Div. of Pharmacology, Food and*

Drug Administration, Federal Security Agency, Washington 25, D. C. A method has been developed for the estimation of small quantities of benzene hexachloride. It is based upon the conversion of the benzene hexachloride to 1,2,4-, 1,2,3- and 1,3,5-trichlorobenzene by alkaline hydrolysis. Of the mixture of trichlorobenzenes formed approximately 82% is the 1,2,4-trichlorobenzene which has a characteristic spectral absorption curve in the ultraviolet region. Advantage was taken of these facts in developing a quantitative method. The method consists of the intial extraction of the benzene hexachloride with ether from the material to be examined, and the conversion of the benzene hexachloride to the 1,2,4-trichlorobenzene by refluxing with 1N methanolic potassium hydroxide for one hour. Purification of the trichlorobenzene is accomplished by extracting with hexane and washing with water, and when necessary by the use of magnesium oxide or alumina chromatographic columns. The quantity of the trichlorobenzene is estimated by measurement of its optical density at 286 $\mu\mu$. Spectral absorption due to other substances is eliminated by applying a formula for the resolution of a two component color system. In this manner as little as 100 μg/total sample may be determined in biological tissues, in spray residues on spinach and cabbage, and in animal laboratory diets.

Isolation of biochemically deficient mutants of bacteria by means of penicillin. BERNARD D. DAVIS. *U. S. P. H. S., Tuberculosis Research Laboratory, Cornell Univ. Medical College, New York City.* Mutants with increased nutritional requirements have been difficult to isolate efficiently because any medium enriched to permit growth of the mutants will also foster growth of non-mutant organisms. A method of selectively isolating such mutants from large populations suggested itself on the basis of the reports that penicillin sterilizes bacteria only under conditions which permit growth. Mutants were therefore exposed to penicillin in minimal medium in which they were unable to grow, and were found to survive while the predominant non-mutant population was eliminated. This screening technique proved to be very efficient when applied to ultraviolet irradiated *E. coli*, and made it possible in a short time to isolate mutants with individual requirements for almost all the vitamins and naturally occurring amino acids, and less specific requirements for purines or pyrimidines, as well as requirements for unknown factors in yeast extract. By testing the syntrophic effect of certain mutants on others grown adjacent to them on solid media, it has been possible to demonstrate several instances in which mutants with a common requirement are blocked at different stages in the synthesis of the nutrilite (arginine, proline, histidine, phenylalanine plus tyrosine, thiamine). Those mutants which visibly feed others must secrete into the medium considerable amounts of a stable compound which is used in biosynthesis.

Peptides: metabolic and structural intermediates in protein metabolism. CLARA L. DEASY (by invitation), HENRY BORSOOK, ARIE J. HAAGEN-SMIT (by invitation), GEOFFREY KEIGHLEY (by invitation), and PETER H. LOWY (by invitation). *Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, Calif.* A large peptide (designated Peptide A) was isolated by starch chromatography from the liver of albacore, beef, guinea pig, hog, horse, lamb and rat and from Witte's peptone. The peptide isolated from different sources had (within the limits of analytical accuracy) the same amino acid composition. The following amino acids have been demonstrated in it: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tryptophane and tyrosine. Guinea pig liver was found to contain a number of other peptides. After incubating the liver homogenate with C^{14}-labeled glycine, leucine or lysine the labeled amino acids were extensively incorporated into Peptide A. In addition leucine is rapidly incorporated into one other peptide and more slowly into another. Labeled glycine (or an amino acid derived from it) was rapidly incorporated into two other peptides. The peptides into which labeled glycine, leucine and lysine are incorporated are normally present in guinea pig liver. They and free amino acids increase during incubation of liver homogenate, more at *p*H 6.0–6.5 than at 7.0–7.5. The incorporation of glycine and leucine is much faster into the peptides than into the proteins. This fact and the finding of the same peptide (Peptide A) in liver and as a major product of the peptic digestion of fibrin (Witte's peptone), are evidence that peptides are metabolic as well as structural intermediates in protein metabolism.

Studies of nuclei isolated from normal rat and rabbit liver by a modified Behrens technique. ALEXANDER L. DOUNCE, GARSON H. TISHKOFF (by invitation), SHIRLEY R. BARNETT (by invitation) and RICHARD M. FREER (by invitation). *Dept. of Pathology and Biochemistry, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* Nuclei were prepared from normal rat and rabbit liver by a modification of the method of Behrens in order to avoid the use of aqueous solvents. The desoxyribonucleic acid of the Behrens nuclei was difficultly extractable, and was at least 50% lower in concentration than the desoxyribonucleic acid of nuclei prepared by aqueous solvents at *p*H 6.0. This finding apparently is due to the loss of nucleic material other than nucleic acid from the latter type of nuclei during their preparation. Arginase, aldolase, catalase, cytochrome oxidase, esterase

and lipase were present in the isolated nuclei. Precise quantitative measurements of the enzyme activity of Behrens type nuclei are difficult to carry out because of agglutination of the nuclei near *p*H 7.0. Free amino acids were extracted from the nuclei with 76% alcohol and were studied by the method of paper chromatography. The pattern yielded by the nuclei appears to be identical with that yielded by the whole tissue. The spots of maximal intensity are those due to glutamic acid, glycine, alanine, leucine plus isoleucine, β-alanine, and probably glutamine, and also to a spot thought to be due to taurine. Aspartic acid, serine, and valine are present in easily detectable but lower amounts, while most of the essential amino acids were detectable only in trace amounts. Glutathione was uniformly detected in nuclei. Its chromatographic detection in whole tissue was more difficult, perhaps because of greater instability in cytoplasm. Glutathione was found to be about equal in concentration in nuclei and whole tissue by chemical titration with iodate.

Hormonal influences on cytochrome *c* metabolism and liver regeneration. DAVID L. DRABKIN. *Dept. of Physiological Chemistry, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* Thyrotoxicosis, induced by thyroxine administration to rats, increased the concentration and content of cytochrome *c* in their tissues (Drabkin, D. L., *Federation Proc.*, 7: 151, 483, 1948). In a continuation of these studies it has been found that, despite the higher concentration of liver cytochrome *c* and PNA in such thyroxinized animals, instead of the usual increase in cytochrome *c* and PNA observed in liver regenerating after partial hepatectomy, there occurred a reduction in the concentration of these constituents, accompanied by an appreciably reduced liver regeneration (43.9 in the thyrotoxic as against 73.9% in the normal). After bilateral adrenalectomy, performed at the time of liver lobectomy in rats on a high (31%) protein diet, liver regeneration in 14 days was consistently greater than that of normal controls and of thyroidectomized animals (89.7 in adrenalectomized, 73.9 in normal, and 65.2% in thyroidectomized). Excluding liver tissue, completely adrenalectomized rats, like thyroidectomized animals, had significant decreases in the cytochrome *c* concentration and content of heart, kidney and skeletal muscle.

Electrophoretic comparison of plasma and body fluid proteins in certain diseases. ROBERT L. DRYER (by invitation), JACQUELINE H. BUDDE (by invitation), W. D. PAUL (by invitation), and JOSEPH I. ROUTH. *Depts. of Biochemistry and Medicine, College of Medicine, State Univ. of Iowa, Iowa City, Iowa.* We have compared the protein components of plasma and body fluids from patients with a variety of clinically diagnosed illnesses. Blood samples were withdrawn whenever it was necessary to perform thoracentesis, paracentesis, or joint aspiration. Protein components of the corresponding samples were determined by electrophoresis in a single center section cell. Samples were withdrawn from a number of the subjects at intervals over a considerable period of time. Ten patients with various cardiovascular diseases were studied, and in general it was found that the paracentesis and thoracentesis fluids showed a distribution of protein components which closely paralleled that of the plasma. Both the plasma and the fluid showed a marked drop in albumin with a corresponding increase in globulins, especially γ-globulin. A urine sample from one subject with marked decompensation gave a protein distribution which almost exactly matched the plasma. Eleven patients with neoplastic conditions ranging from gastric carcinoma to reticuloendothelial hyperplasia were also studied. Again the most outstanding characteristic was the marked reduction in albumin. In a case of multiple myeloma a component migrating faster than albumin was found in plasma and urine. In several carcinoma cases the α-globulins rather than the γ-globulin fraction showed a greater increase in the plasma and fluids.

Non lability of choline methyl and the role of choline oxidase in transmethylation. JACOB W. DUBNOFF. *California Institute of Technology, Pasadena, Calif.* The methyl groups of choline are not labile but become available for methionine formation from homocysteine only if the animal can oxidize choline to betaine. *In vitro* studies show that methionine formation is rapid from betaine, dimethylthetin, and certain derivatives of dimethylthetin both aerobically and anaerobically in all animals tested, but choline is effective only in those animals which have choline oxidase. While the aerobic synthesis of methionine from choline in these animals approaches that from betaine, anaerobic methionine formation from choline is relatively small or absent. Rabbit, guinea pig and chick, which do not have choline oxidase, do not form methionine from choline in liver and kidney homogenates aerobically or anaerobically. Inhibition of aerobic methionine formation from choline in rat by choline analogues parallels their effects on choline oxidase. There is no significant aerobic methionine formation from choline at *p*H 6.7 where its oxidation proceeds only to the aldehyde stage. The importance of choline oxidase may be to enable the animal to divert choline methyl into the general transmethylation scheme.

Thiocyanation of tyrosine. MARIE EASTERWOOD (by invitation) and JOHN L. WOOD. *Dept. of Chemistry, School of Biological Sciences, University of Tennessee, Memphis, Tenn.* Thiocyanate ion has been shown to interfere with the metabolism of tyrosine in the thyroid gland. This physiological

activity has been suggested to be an interference of the 'metabolic antagonist' type. Studies on thyroid glands from animals given potassium thiocyanate labeled with radioactive sulfur indicate that the thiocyanate ion itself combines chemically with protein. The data, and the chemical properties of tyrosine and thiocyanogen, are consistent with the hypothesis that administered thiocyanate may react with tyrosine, by a mechanism similar to iodination, to form thiocyano derivatives. This point of view is supported by the thiocyanation of tyrosine *in vitro*. Tyrosine reacts with thiocyanogen (corresponding in state of oxidation to free iodine) in glacial acetic acid to form thiocyanotryosine. The compound has been characterized by ultimate analysis, optical activity, absorption spectrum, and by the formation of derivatives.

On the preparation and preservation of Fraction I from human plasma. JOHN T. EDSALL, JOHN D. FERRY, and PETER R. MORRISON (by invitation). *Dept. of Physical Chemistry, Harvard Medical School, Boston, Mass.* In the preparation of Fraction I (the fibrinogen-containing and most insoluble major fraction of plasma as separated by ethanol precipitation, cf. Cohn, et al., *J. Am. Chem. Soc.*, 68: 459, 1946) from human plasma previous conditions of blood processing are particularly important and may be critical. Both short handling times and quick cooling of the blood are needed to insure a stable product. The optimal conditions for the separation from plasma are *p*H 7.3, 8% ethanol and −3° C. Although the gross purity of the fraction (60–65% fibrinogen) is not sensitive to *p*H, components are precipitated below *p*H 7.0 which greatly reduce the stability of the product. Fraction I is most conveniently stored as frozen precipitate, but is much less stable either in this form or as frozen solution than when stored in the dry state. In the latter form, and stored under vacuum, it has been successfully kept at room temperature for more than 4 years. It is less stable in air and more stable at low temperatures. Solutions of fibrinogen are less stable than albumin or globulin at both ordinary and elevated temperatures. They are more like the latter in their response to stabilizing agents. Testing procedures for Fraction I have been followed on a considerable series of bottled 'commercial' preparations. Criteria included total and relative amounts of fibrinogen, *p*H and buffering capacity, clotting times with thrombin and with calcium, stability of the solutions at 25°, 37° and 45°, and of a clot at 37°C.

Estimation of ergosterol with activated glycerol dichlorohydrin. ROMOLA ETTINGER (by invitation) and Albert E. Sobel. *Polytechnic Institute and Jewish Hospital, Brooklyn, N. Y.* The reaction between Ergosterol and GDH (glycerol dichlorohydrin) was studied spectrophotometrically. Activated GDH (Sobel and Werbin, *Anal. Ed.*, 18: 570, 1946) containing 4% acetyl chloride produces a greenish yellow color suitable for the determination of ergosterol. Cholesterol and 7-dehydrocholesterol do not interfere. Ergosterol values obtained on brewer's yeast are similar to those with the $SbCl_3$ method. (Lamb, Mueller and Beach, *Anal. Ed.*, 18: 187, 1946) but lower than the less specific ultraviolet absorption method. A new method of extraction was developed which is simple, reliable, requires small samples (i.e. 25 to 50 mg. brewer's yeast) and permits rapid, simultaneous determinations. Specimen is placed in a glass stoppered tube, saponified with 1N alcoholic KOH, extracted three times with petroleum ether in this tube, extracts evaporated to dryness with N, dissolved in 1 ml. of chloroform, treated with 4 ml. of GDH reagent and after 20 minutes read in the spectrophotometer at 410 mμ. Quantitative recoveries were made of known amounts of ergosterol added to the brewer's yeast.

The effect of ethionine on fat metabolism and on protein formation. E. FARBER (by invitation), M. SIMPSON (by invitation), and H. TARVER. *Div. of Biochemistry, Univ. of California Medical School, Berkeley, Calif.* When DL-ethionine is injected into fasted rats fat rapidly appears in the liver so that in 12 hours the fat content is 14% (Control 6%). Fat continues to accumulate and the animals die within the next 24 hours. The appearance of fat in the liver is prevented by giving D, or L-methionine but not by other amino acids such as L-lysine, DL-valine, glycine, or L-alanine in equimolar doses. Choline lowers the fat content to only 12.5%. Orally administered sucrose (5 gm.) or glucose prevents the fat accumulation. The condition is either cured or at least markedly improved by giving methionine 12 hours after the ethionine. In short term *in vivo* experiments in which ethionine is injected along with labeled methionine (S^{35}) the uptake of the label into liver protein is greatly reduced during the next 3 hours (26 and 53% in different experiments). This inhibition of protein formation is reversed by equimolar doses of methionine. Sucrose given orally does not significantly effect the inhibition. Ethionine also inhibits the conversion of methionine to cystine *in vivo* and this inhibition is relieved by methionine injected in larger doses than are required to relieve the protein inhibition. Probably ethionine prevents the demethylation of methionine. In liver slices the effects of ethionine on protein metabolism are practically duplicated. The inhibition of methionine uptake depends on the ethionine concentration and is eliminated by methionine—best by the L-isomer. Ethionine also inhibits the uptake of glycine both *in vitro* and *in vivo*. Methionine *in vivo* relieves the inhibition.

Quantitative paper chromatography of penicillins. II. Development of buffered strips wi

organic solvent. OLIVER B. FARDIG and NANCY D. BREED (introduced by S. B. BINKLEY). *Research Division, Bristol Laboratories, Inc., Syracuse, N. Y.* A paper strip chromatographic procedure has been developed whereby the commonly known penicillins were completely separated from one another by developing procaine citrate-buffered strips with amyl acetate for 15 to 18 hours. Filter paper strips were buffered the first part of their length with *p*H 5.2 procaine citrate, spotted with 0.5 to 2.5 U of penicillin, equilibrated in a chamber saturated with respect to water and amyl acetate vapor, and developed in the same chamber. The dried developed chromatograms were placed on *Staph. aureus*-inoculated trays which were then incubated to reveal the position of the individual penicillins. Two chromatograms of different concentrations of the same solution were placed on the same tray. Photographs of the incubated trays were made to facilitate calculation. The amount of penicillin present was determined from measurements of the maximum width of each zone on the photograph. Analysis of penicillin mixtures of known composition gave results in excellent agreement with the known values. No interference was observed in the determination of the penicillins in broth. The rate of movement of the penicillins along the strip increased in the order: *p*-hydroxybenzylpenicillin, benzylpenicillin, 2-pentenylpenicillin, pentylpenicillin, and *n*-heptylpenicillin. The usual spread of the fast-moving *n*-heptylpenicillin was prevented by allowing this penicillin to move beyond the buffered portion of the paper strip. The separation of the penicillins, their rate of movement, and their compactness on the strips was influenced by the buffer, the solvent, and the conditions of development.

Glycolysis and the Pasteur effect on the subcellular level. SILVIO FIALA (introduced by DEAN BURK). *National Cancer Institute, Bethesda, Md.* Mouse sarcoma 37, when homogenized in 8.5% sucrose solution, yielded on 7 minutes centrifugation at 20,000xg a pellet of large particles, and after 2-3 hrs. at 20,000xg a microsomial gel. Both fractions when washed and re-suspended in 8.5% sucrose, and after addition of fortifying factors (DPN 4×10^{-4}, ATP 8×10^{-4}, Nicotinamid 32×10^{-4}, Na-pyruvate 8×10^{-3}, $MgCl_2$ 5×10^{-3}, $KHCO_3$ 2×10^{-2}, and glucose 0.5%, at *p*H 7.1) glycolyze HDP but not glucose alone. Use of the Ringer solution results in a lowering of glycolytic activity. Under conditions mentioned there is no oxygen uptake in any fraction. The glycolytic activity of the large granules and microsomial fractions is independent of the presence of oxygen, but a small Pasteur effect in the supernate may occur. When either the large particles or the microsomial pellet is mixed with the supernatant, the anaerobic glycolysis (N_2/CO_2) of the combination is equivalent to the sum of the glycolysis of each fraction run separately. However, under aerobic conditions (O_2/CO_2) the glycolysis of the combined fractions is lower than of any single fraction. Mixing the particulate elements with the supernatant thus results in the appearance of a pronounced Pasteur effect. Normal mouse liver particulates and the supernatant fluid (after removal of microsomes) showed the same mutual interaction in regard to Pasteur effect but quantitative differences occurred. In the attempt to determine the centrifugal force sufficient to concentrate the remaining glycolytic activity in the supernatant fluid (after removal of formed elements) in the experiments done with Dr. G. Kegeles (National Cancer Institute) it has been found that after a run of 4 hours at 164,000xg the activity in the bottom layer was 2x that of the top layer of the centrifuged sample.

Influence of estrogenic hormones on b-glucuronidase in the human. WILLIAM H. FISHMAN and LESTER D. ODELL (by invitation). *Depts. of Surgery and Biochemistry, Obstetrics and Gynecology, Univ. of Chicago, Chicago, Ill.* In previous work a relationship between the metabolism and action of the estrogenic hormones and b-glucuronidase was indicated. In castrate female mice, the uterine glucuronidase responds by an increase in its activity following the injection of estrogenic substances. In pregnant women, the serum b-glucuronidase becomes elevated. Additional experiments have now been done in humans with a view to exploring the extent of the relationship of estrogen metabolism to b-glucuronidase activity. The following observations have been made. In 15 post-partum women receiving no medication, whether lactating or non-lactating, the serum b-glucuronidase falls from approximately 500 U/100 cc. regularly to non-pregnancy levels by the sixth day after parturition. On the other hand, the decline in glucuronidase was much smaller and more gradual in post-partum women receiving stilbestrol (5 to 25 mg/day for periods of 10 days). Endometrial biopsies were obtained from 33 women at various days of the normal menstrual cycle and assayed for B-glucuronidase activity. The enzymic activity increased during the first two-thirds of the menstrual cycle, followed by a decline, particularly in the last three days of the cycle. The presence or absence of progestational endometrium seemed to bear little reference to these changes. From these and other observations, it is felt that estrogen may be at least one of the factors which influence the b-glucuronidase level in the serum and in uterine endometrium.

Amylase and lipase in the intestinal lymph of the rat. E. V. FLOCK and J. L. BOLLMAN. *The Mayo Foundation, Rochester, Minn.* Lymph was collected continuously from the small intestine of the rat after cannulation of the main lymphatic trunk with polyethylene tubing. The rats had been fasted

24 hours prior to the operation, and on the day following received 2 meals of either a fat-free or high-fat diet by stomach tube. Lymph collected overnight before the first meal and at 2, 4 and 18 hour intervals thereafter was analyzed for amylase and lipase activity. The amylase was determined by the copper reduction method of Somogyi and the lipase (tributyrinase) by the method of Goldstein and Roe. The amylase activity of the intestinal lymph was lower than that of the plasma in the fasting rat. The response to feeding was slight and variable. If an increase was found, the level reached was generally lower than that of the plasma of the fed rat. The average hourly output of amylase in the lymph increased markedly only during the first 2 hours after feeding and this was at least in part due to the increased rate of flow. Higher values for tributyrinase activity were found throughout the 24 hour feeding period studied in the intestinal lymph of rats fed the high-fat diet than during fasting or after feeding of the fat-free diet. The average hourly output of lipase in the lymph of rats fed the high-fat diet was also increased throughout the 24 hour period whereas in rats fed the fat-free meals an increase was found only during the first 2 hours after feeding.

A new P-free and S-free brain lipide. J. FOLCH and S. ARSOVE (by invitation). *McLean Hospital, Waverley, Mass. and Harvard Medical School, Boston, Mass.* A new lipide has been found to be present in grain. It appears to be P-free and S-free and to have as a constituent an as yet unidentified primary amine which becomes water-soluble after short-time acid hydrolysis. The new lipide has been partially purified. The preparation obtained is still contaminated by phosphatides (0.15 per cent P) and sulfatides (0.25 per cent S). The constituents of the new lipide appear to be sphingosine (1.1 per cent sphingosine N) or a sphingosine-like substance, carbohydrate (20 per cent as galactose), an unidentified primary amine (1.1 per cent water-soluble NH_2 after acid hydrolysis) and unsaponifiable substances (only small amounts of fatty acids are obtained from it after saponification). The constituent amine is not neuraminic acid (Klenk, E., *Ztschr. f. physiol. Chem.* 268: 50, 1941 and it is linked to the rest of the molecule by its NH_2-group, since the intact lipide does not exhibit any free NH_2-group. The new lipide is soluble in acetic acid, and in $CHCl_3$:CH_3OH mixtures at room temperature and insoluble in the latter at $-10°$. The method of isolation which has yielded a partially pure product is based on these solubilities. The new lipide appears to be a contaminant of a large number of cerebroside and sphingomyelin preparations that have been analyzed.

Cholesterol studies on lyophilized chicken serum. J. C. FORBES and G. H. L. DILLARD (by invitation). *Dept. of Biochemistry, Medical College of Virginia, Richmond, Va.* The serum cholesterol of both male and female chickens of varying ages has been fractionated in a manner previously described (*Proc. Soc. Exper. Biol. & Med.* 68: 240, 1948). The results indicate that a large percentage of the cholesterol of lyophilized serum from old hens is extracted by cold chloroform in 3 hours, behaving in this regard very similarly to the serum of nephrotic patients and hypercholesterolemic rabbits. Old hens, however, show this high degree of cholesterol extractability even though the total cholesterol concentration is normal. The sera of young chickens and old roosters show a low value for this 'readily extractable fraction.' Studies on the effect of a high fat diet on these fractions in roosters of various ages will be presented also.

Synthesis of some benzoylamino acid anilides as catalyzed by papain. SIDNEY W. FOX, FREDERICK N. MINARD (by invitation), HARRY WAX (by invitation), CORNELIUS W. PETTINGA (by invitation), and JACQUETTA STRIFERT (by invitation). *Chemical Laboratory, Iowa State College, Ames, Iowa.* The synthesis of anilides from 4 benzoyl-DL-amino acids and aniline, as catalyzed by papain, has been studied in consolidated experiments. The alanine and leucine derivatives were considerably preferred in reactivity over glycine and valine. Benzoylglycine showed relatively moderate reactivity and benzoylvaline barely reacted. Benzoylvaline was the only valine derivative of several (formyl, acetyl, 3,5-dinitrobenzoyl, benzoyl, phthaloyl, and *o*-carboxybenzoyl)-DL-valine, which gave the acylamino acid anilide. The last 2 valine derivatives yielded N-phenylphthalimide, which was also the product isolated from *o*-carboxybenzoyl-DL-valine and aniline in either the absence or presence of enzyme. The relative non-reactivity of benzoylvaline was not due to the presence of the D-isomer, as shown by approximately equal reactivity of the L-component in the L- and DL-forms. These results are of interest in relation to the observation of Roche and Mourgue that papain liberates much more leucine than valine from casein. Roche and Mourgue ascribed this behavior to position of residues in the substrate molecules; in the present work the results can be explained on the basis of greater reactivity of leucine bonds than of valine bonds in papain-catalyzed systems. Hydrolytic data for papain and compounds containing the same residues has been obtained. The retarding effect of various substances, such as iodoacetic acid, P-quinone, and 2,3,5-triphenyltetrazole, upon some anilide syntheses has been studied.

Mechanism of reversible inactivation of lysozyme by iodine and sulfite. H. FRAENKEL-CONRAT. *Western Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of*

Agriculture, Albany, Calif. Meyer *et al.* (*J. Biol. Chem.* 113: 303, 1936) showed that lysozyme loses and regains activity upon treatment with iodine and sulfite, respectively. It has now been found that the effect of a great excess of iodine is partly reversible only after 10–120 minutes at or above neutrality. Reactivation was achieved also with thioglycol and was found associated with a loss in bound iodine, equivalent to 60–100% of the histidine content of lysozyme. Proteins of greatly varying histidine contents, when treated with iodine and sulfite under similar conditions, contained reversibly bound iodine in proportion to their histidine contents. From this and other evidence it is concluded that the reversibly inactivated derivative is formed first and probably contains a labile N-iodohistidine residue, whereas the irreversibly inactivated derivative is formed later by rearrangement of the N-iodohistidine into a stable C-iodo form. The concomitant iodination of some phenolic groups appeared to cause little inactivation, but when over 2 iodine equivalents (per mole lysozyme) were introduced into these groups, the activity was greatly lowered. The incipient oxidative destruction of tryptophan residues (less than 1 per mole) appeared reversible by early reduction. Lysozyme contains no —SH group.

Pteroylglutamic acid deficiency in the dog as produced by 'x-methyl' pteroylglutamic acid. A. L. FRANKLIN (by invitation), T. H. JUKES, E. L. R. STOKSTAD, and M. BELT (by invitation). *Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.* Dogs were kept on wire floors in individual cages and were fed a purified diet with pteroylglutamic acid (PGA) omitted and with a crude PGA antagonist, 'x-methyl' PGA, added. The dogs grew slowly or lost weight and developed a condition marked by emaciation, alopecia, anemia and ulceration of the skin. A slight hemopoietic response with a small reticulocyte rise was obtained when liver extract was injected. However, when PGA was administered a marked response was obtained together with a prompt gain in weight and with growth of hair in the denuded areas. Dogs appeared normal when they received the diet with the antagonist omitted or with sufficient PGA added to reverse the antagonist as judged from experiments with other species.

Observations on the equilibrium between glycine and glycylglycine in the presence of liver peptidase. IVAN D. FRANTZ, JR., ROBERT B. LOFTFIELD, and ANN S. WERNER (introduced by FRITZ LIPMANN). *Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard Univ., Massachusetts General Hospital, Boston, Mass.* Methods have been worked out which permit the analysis of equilibrium mixtures of glycylglycine and glycine. These are based on the use of long-lived radioactive carbon, for the detection and measurement of small amounts of the reactants, and chromatography of starch, for their separation. In a typical experiment, labeled glycine is incubated at 38° with a peptidase preparation made from rat liver. The *p*H is held at approximately 7.6 by means of a borate buffer. After 48 hours the reaction is stopped. Glycylglycine is added as carrier, and the reaction mixture is placed on a column of potato starch. 2:1 normal propanol-water is passed through the column, and the effluent is analyzed by the photometric ninhydrin method of Moore and Stein (*J. Biol. Chem.* 178: 367, 1948). The glycine and glycylglycine peaks are each pooled and analyzed for radioactivity. With glycine concentrations as high as 1 molar, the experiment has also been carried out with the addition of carrier, allowing confirmation of the results independent of the use of radioactive carbon. This possibility exists because of the high resolving power of the column, and its large capacity for glycine. Thus, 30 mg. of glycine may be completely separated from 60 μg. of glycylglycine, formed during the reaction. The free energy of the peptide bond as determined in this way is in fair agreement with the value calculated by Borsook and Dubnoff, based on thermal measurements (*J. Biol. Chem.* 132: 307, 1940).

Purification and properties of ovomucoid. E. FREDERICQ (by invitation) and H. F. DEUTSCH. *Depts. of Physical and Physiological Chemistry, Univ. of Wisconsin, Madison, Wis.* The solubility of ovomucoid in trichloracetic acid solution has been utilized for its isolation. In an egg white system of 5% sodium trichloracetate and *p*H 3.5, the major portion of the ovomucoid remains in solution while the other proteins are precipitated almost quantitatively. The ovomucoid is then recovered by precipitation at an ethanol concentration of 65% and temperature −7°C. The product consists of 2 components which are closely related electrophoretically. The major component comprises from 90–95% of the total protein. It may be separated in relatively pure form from 1% solution by several precipitations at *p*H 3.2, ionic strength 0.01, and ethanol concentration 42%. On electrophoresis this protein behaves as a single component over a considerable *p*H range and has an isoelectric point at *p*H 3.8. In experiments performed in the region of the isoelectric point some electrophoretic inhomogeneity is evident. Its electrophoretic mobility in diethylbarbiturate buffer at *p*H 8.6 and ionic strength 0.1, is 4.3×10^{-5} cm.^{2} volt^{-1}sec^{-1}. Ovomucoid which has been isolated by electrophoresis gives a sedimentation constant of s_{20w} = 2.9s. This value is also obtained with the ovomucoid separated by fractionation. Preliminary carbohydrate analyses of the crude ovomucoid show a level of approximately 10% calculated as mannose.

Competitive antagonists of thyroxine and structurally related compounds. EARL FRIEDEN (by

invitation) and RICHARD J. WINZLER. *Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles, Calif.* Thyroxine antagonism by several new compounds has been observed using the amphibian metamorphosis test system described by Frieden and Winzler (*J. Biol. Chem.*, 176: 155, 1948). The competitive nature of these inhibitors was shown by the relative constancy of the molar ratio of inhibitor to thyroxine required for reduction of the thyroxine effect by 50%, and by adherence of the data to the more rigorous equation of Lineweaver and Burk (*J. Am. Chem. Soc.*, 56: 658, 1934). Reduction of the thyroxine effect by 50% by O-benzyl-3,5-diiodo-DL-tyrosine was achieved with molar ratios of 37, while the N-acetyl derivative, described by Woolley (*J. Biol. Chem.*, 164: 11, 1946), required a ratio of 550. The benzyl, p-nitrophenylethyl, and methyl ethers of 3,5-diiodo-4-hydroxybenzoic acid required ratios of 8, 75, and 150 respectively for similar effects. Specificity of these antagonisms was indicated by the absence of any inhibition of thyroxine by structurally related compounds, goitregens, and other substances. Inhibition of the thyroxine-like activity of 3,5-diiodo-4-(3′,5′-diiodo-4′-hydroxyphenoxy)benzoic acid was obtained at the same molar ratios with several of the above thyroxine antagonists. However, no inhibition of the activity of 3,5-diiodo-4-(4′-hydroxyphenoxy)aniline was observed at very high molar ratios. Assuming that thyroxine-like active compounds act by serving as a prosthetic group or coenzyme for some enzyme system or systems, these data lend support to suggestions that the side chain of thyroxine serves as an enzyme-associating locus while the ortho-diiodo hydroxy phenyl group acts as a 'functional' locus of the molecule.

Liver regeneration in the adrenalectomized rat. CHARLES E. FRIEDGOOD (by invitation), HARRY M. VARS and JOAN W. ZERBE (by invitation). *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* In continuing previous studies of N metabolism and liver protein regeneration male Wistar rats have been adrenalectomized at the time of partial hepatectomy. Saline drinking water was available throughout the 2-week postoperative period. Total net N balances and new liver protein formed have been determined. Adrenalectomy reduced the amount of liver protein regeneration in rats both protein-fed and protein-depleted before operation. Whole adrenal extract administered to such rats restored their ability to regenerate new liver protein. Rats, protein-depleted for 2 weeks and subjected to the double operation, were given daily injections of desoxycorticosterone acetate, 11-dehydrocorticosterone acetate and 11-dehydro, 17-hydroxycorticosterone (acetate) during the 2 week postoperative period. At the dose levels used, all stimulated hepatic protein metabolism and caused the same or greater regeneration of new liver protein than occurred in animals with intact adrenals. DOCA caused the greatest increment, accompanied by evidence of hormonal overdosage. 11-dehydrocorticosterone appeared to be slightly more active than 11-dehydro, 17-hydroxycorticosterone.

Flavoenzyme catalysis. Sulfhydryl character of the d-amino acid oxidase. W. R. FRISELL (by invitation), E. C. WEINBACH (by invitation), R. C. COWGILL (by invitation), and LESLIE HELLERMAN. *Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore, Md.* In earlier studies (1940–42) we observed that the activity of the d-amino acid oxidase of lamb kidney is suppressed by *p*-chloromercuribenzoate (Hellerman in *Cold Spring Harbor Symposia on Quantitative Biology*, 7: 165, 1939) and by porphyrindine. Notes have appeared from other laboratories on effects of sulfhydryl-binding agents upon such oxidases. Of outstanding biochemical importance is the role of 'available' protein-sulfhydryl groups essential to the activity of certain enzymes. Although *p*-chloromercuribenzoate and also iodosobenzoate can attack the separated protein of lamb d-amino acid oxidase, and, less reversibly, pig kidney oxidase, it is demonstrated that these reagents in low concentration may function instead as competitive inhibitors for the substrate. It is essential, in these studies, to recognize that certain flavoenzymes dissociate; further, that the aforementioned 'sulfhydryl-reagents' function as such, and, independently, as typical unsaturated anionic inhibitors (*Transactions of the Second Conference, Biological Antioxidants*, 78, 1947. Any labile sulfhydryl groups of these flavoenzymes appear to be 'protected' by FAD. Certain reagents attack protein-sulfhydryl irreversibly, in the thermodynamic sense. Cogent evidence is revealed in experiments with phenylmercuric acetate. Kinetic studies show that the inhibition observed here is not substrate-competitive. The action of authentic competitive inhibitors, e.g. cinnamate ion, may be superimposed upon that of the mercurial. The results in no way support a concept that enzyme-sulfhydryl here is concerned directly with substrate binding or is involved in substrate 'activation.'

Preparation of deuterated steroids. DAVID K. FUKUSHIMA (by invitation) and SEYMOUR LIEBERMAN. *Dept. of Steroid Biochemistry, Sloan-Kettering Institute for Cancer Research, New York City.* For the purpose of studying some problems in steroid metabolism we have investigated the preparation of deuterium labeled steroids. Two general methods for the introduction of stably bound deuterium into Δ^5-steroids have been developed. The first of these involved halogenation with bromosuccinimide at C_7 of a steroid ester followed by catal-

ic reduction with deuterium in the presence of palladium or deuterized Ranney nickel. An alternate procedure, which was advantageous in that 2 deuterium atoms were incorporated, consisted in the conversion of a Δ^5-7-ketosteroid ester to the corresponding mercaptol by condensation with ethanedithiol. Upon desulfurization with deuterized Raney nickel, the cyclic thioacetal was converted to the Δ^5-7,7-d_2-steroid. By these methods deuterium labeled cholesterol and Δ^5-androstenediol-3β,17α-acetate-3,benzoate-17, an intermediate in the synthesis of testosterone, were prepared.

Hepato-renal factors in circulatory homeostasis. XXIV. Conditions leading to aerobic formation of VDM by liver. ROBERT F. FURCHGOTT, BENJAMIN W. ZWEIFACH (by invitation) and EPHRAIM SHORR (by invitation). *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* Previous experiments from this laboratory have shown that normal liver slices form the vasodepressor, VDM, only under anaerobic conditions; on aerobic incubation, VDM formation is inhibited and VDM added to the incubation medium is inactivated. In contrast, livers from animals in irreversible shock sustain a profound impairment in the capacity to inactivate VDM on aerobic incubation. A similar impairment is shown by normal liver slices following prior exposure to anaerobiosis. In a recent extension of these studies, it has been observed that liver slices from dogs in irreversible shock not only lose their capacity to inactivate VDM aerobically, but also continue to form VDM under aerobic conditions. This was also the case with normal liver following an appropriate period of anaerobic exposure; anaerobic incubation of normal liver (dog, rabbit) for one hour at 37.5°C. usually results in the continued elaboration of VDM on the restoration of aerobic conditions. With both types of liver preparations, the rate of aerobic VDM formation is comparable to that of normal liver slices under anaerobic conditions. Respiratory studies of liver slices, treated so as to form VDM aerobically, revealed no relation between the loss of ability to inhibit aerobic VDM formation and changes in oxygen consumption. The significance for shock of this derangement in VDM metabolism resides in the fact that the temporary restoration of aerobic conditions within the liver by transfusions would not, per se, prevent the continued elaboration of VDM and the persistence of the decompensatory effects of this vasodepressor principle on the peripheral circulation.

Chloride diarrhea and systemic alkalosis in potassium deficiency. LYTT I. GARDNER, ELSIE A. MACLACHLAN, MARY L. TERRY, JANET W. MCARTHUR and ALLAN M. BUTLER (introduced by RALPH W. MCKEE). *Children's Medical Service, Massachusetts General Hospital, and the Dept. of Pediatrics, Harvard Medical School, Boston, Mass.* Severe potassium deficiency was produced in 160 to 175-gm. rats by use of a diet containing 0.003% K and 0.7% sodium. K added to the diet produced excellent growth in controls. Rats fed the deficient diet gained weight slowly for 35–40 days, then diarrhea began, followed by anorexia, wasting and death between 60 and 120 days. Muscle K was reduced almost 50%. Hypochloremic alkalosis was present. Metabolic studies while on the deficient diet showed negative K and Cl, and positive Na balances. A single injection of 1.0 mEq. K as K_2HPO_4 produced transitory reversal of these balances and temporarily arrested the diarrhea and anorexia. The Cl and Na balances lagged 3–4 days in their reversal. Fecal excretion of Cl progressively diminished for 6 days post-injection. Urinary Cl decreased for 3 days post-injection, then abruptly increased on the 4th day, coincident with marked Na diuresis. This may reflect the extrusion of intracellular Na, covered in the urine by the anion Cl, cf. Conway and Hingerty, *Biochem. J.* 42: 372, 1948. On the deficient diet daily Cl excretion was double that on the control diet. There was 4 times as much Cl in the feces of the deficient animal as in the feces of the control. Even during diarrhea there were twice as many mEq. of Cl as Na in the feces of the deficient animal. The reported cases of congenital alkalosis with diarrhea, and 2 cases of ulcerative colitis seen at this hospital, show many metabolic similarities to the above findings, which suggest deficit of intracellular K. The etiologic role played in these syndromes by adrenal overproduction of the 11-desoxycorticosteroid (DOCA) type of hormone, known to produce K deficiency when injected, is under investigation.

Inhibition of hyaluronidase by blood serum and bile constituents. DAVID GLICK, LEE W. WATTENBERG (by invitation) and ERICK Y. HAKANSON (by invitation). *Dept. of Physiological Chemistry, Univ. of Minnesota Medical School, Minneapolis, Minn.* In an attempt to determine the origin of the hyaluronidase inhibitor in blood serum, 29 organs and bile, urine, and amniotic fluid of the rabbit, in addition to human saliva, milk, and spinal fluid, and hog synovial fluid were tested. No inhibitory activity was found by the viscosimetric method in any of them exceept bile and urine. The inhibitor in the latter fluids was heat stable and active in the presence of phosphate, thus differentiating it from that in serum. A variety of hemoglobin derivatives, bile salts, and sterol hormone conjugates have been found to inhibit hyaluronidase. The additive value of the individual inhibitions of any one of the compounds and that of serum was always greater than the observed inhibition of a combination of the two. The data would indicate that the hemoglobin derivatives and bile salts inhibited the serum inhibitor, and that serum also reduced the inhibition

of these compounds. Exceptions to this mutually antagonistic effect were observed with certain of the sterol conjugates. Stercobilin was unique in that it did not inhibit hyaluronidase under the conditions used while it did inhibit the serum inhibitor. Studies of the variations of the human serum inhibitor during the menstrual cycle revealed highest levels during actual menstruation and lowest during subsequent and middle phases of the cycle. No significant change was found during pregnancy or labor, but a marked post-partum rise was observed which reached a maximum from the 2nd to 5th days after delivery.

Amino acid composition of casein, α-casein and β-casein. WILLIAM G. GORDON, WILLIAM F. SEMMETT (by invitation), and MYRON MORRIS (by invitation). *Eastern Regional Research Laboratory, Philadelphia, Penna.* The investigation of the comparative amino acid composition of whole casein, α-casein and β-casein previously reported in part (*Federation Proc.* 6: 255, 1947) has been completed. The analytical results, expressed as grams of amino acid yielded by 100 gm. of dry, ash-free protein, follow.

	Whole Casein	α-Casein	β-casein
Glycine	2.7	2.8	2.4
Alanine	3.0*	3.7*	1.7*
Valine	7.2	6.3	10.2
Leucine	9.2	7.9	11.6
Isoleucine	6.1	6.4	5.5
Proline	11.3	8.2	16.0
Phenylalanine	5.0	4.6	5.8
Cystine	0.34	0.43	<0.1
Methionine	2.8	2.5	3.4
Tryptophan	1.2	1.6	0.65
Arginine	4.1	4.3	3.4
Histidine	3.1	2.9	3.1
Lysine	8.2	8.9	6.5
Aspartic acid	7.1	8.4	4.9
Glutamic acid	22.4	22.5	23.2
Amide nitrogen	1.6	1.6	1.6
Serine	6.3	6.3	6.8
Threonine	4.9	4.9	5.1
Tyrosine	6.3	8.1	3.2

* Provisional.

Calculation of the data on the basis of N distribution shows that 101.5% of the whole casein N, 101.8% of the α-casein N and 103.4% of the β-casein N have been accounted for in terms of recognized amino acids and amide groups.

Effect of N,N-dibenzyl-β-chloroethylamine (dibenamine) on blood sugar response to insulin and epinephrine. R. LORIMER GRANT. *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* The subcutaneous injection of dibenzyl-β-chloroethylamine hydrochloride 18–20 hours before the injection of an insulin-containing drug appeared to prolong the hypoglycemic effect of the insulin. Epinephrine injected during the hypoglycemia was significantly inhibited as an antidote to the insulin. Nickerson and Goodman (*J. Pharmacol. and Exper. Therap.* 89: 167, 1947) found that dibenzyl-β-chloroethylamine produced no significant inhibition of th epinephrine-induced blood sugar rise in rabbits We repeated their experiment using a 12 rabbi cross-over test and demonstrated a significant in hibition of the epinephrine-induced blood sugar rise following treatment with dibenzyl-β-chloroethylamine. The effect of dibenzyl-β-chloroethyl amine on the response to insulin is apparently du to inhibition of endogenous epinephrine or othe sympathomimetic factors which increase the rat of glycogenolysis.

Studies on hypoxanthine synthesis in pigeon liver with labeled formic acid. G. ROBERT GREENBERG (introduced by HARLAND G. WOOD). *Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* It previously has been shown that $HC^{14}OOH$ is incorporated into hypoxanthine in pigeon liver homogenates (*Arch. Biochem.* 19: 337, 1948). Further studies have been conducted to elucidate the intermediate steps in this synthesis using $HC^{14}OOH$ to trace the reaction. Approximately 60–70% of formic acid was accounted for in hypoxanthine but almost 100% of the formic acid disappeared during the same period of time. This suggested that the $HC^{14}OOH$ had either entered into other reactions or was present in intermediate compounds involved in the synthesis of hypoxanthine. Small volumes of the reaction mixture were subjected to filter paper chromatography. The paper strips were cut into 0.5 or 1 cm. sections and the radioactivity of each determined. Two distinct radioactive compounds migrated down the paper. One of these has been identified as hypoxanthine and contains the majority of the radioactivity. The other component has a distinctly different absorption spectrum than that of hypoxanthine. The homogenate may be separated into 2 components, the washed particles and the supernatant. Neither fraction shows much activity alone but in combination almost the full synthetic ability is restored. Either component is inactivated by boiling.

Short-term pyridoxine deprivation in two human subjects. LOUIS D. GREENBERG, DAVID F. BOHR (by invitation), HOPE MCGRATH (by invitation) and JAMES F. RINEHART (by invitation). *Dept. of Pathology, Univ. of California Medical School, San Francisco, Calif.* Studies of the blood pyridoxine and the urinary excretion of pyridoxine, 4-pyridoxic acid, xanthurenic acid, tryptophan and N_1-methylnicotinamide were carried out on two human subjects who persisted on a pyridoxine deficient diet for three weeks. The diet consisted of casein, sucrose, corn oil and salts supplemented with the known vitamins excepting pyridoxine, p aminobenzoic acid, vitamin K and tocopherols. By the administration of extra pyridoxine prior to the withdrawal of the vitamin, the blood pyridoxine concentration was increased from initia

values of 1.5 to 2.0 μg. to values of 4.1 to 7.1 μg. %. At the end of the deficient period the levels had fallen to approximately the initial values or lower. The urinary pyridoxine and 4-pyridoxic acid paralleled each other, rising and falling during periods of administration and depletion of the vitamin, respectively. At the end of 2–3 weeks of depletion, xanthurenic acid excretion following 10 gm. doses of DL-tryptophan was markedly increased and returned to low levels after supplementation with pyridoxine (10–15 mg. daily) for one week. The urinary excretion of tryptophan as measured microbiologically was not significantly altered by deficiency, but chemical determination of tryptophan revealed that large quantities of tryptophan or an indole derivative appeared in the urine during the depletion period when test doses of DL-tryptophan were fed. Excretion of the metabolite remained high despite supplementation of the experimental diet with pyridoxine for one week. In confirmation of the work of others, an increased excretion of N_1-methylnicotinamide was observed after the feeding of tryptophan.

Effect of aromatic hydrocarbons on the metabolism of exogenous sulfur amino acids. HELMUT R. GUTMAN (by invitation) and JOHN L. WOOD. *Dept. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis, Tenn.* The changes in urinary sulfur distribution produced by administration of aromatic hydrocarbons to rats were studied after injection of L-cystine or DL-methionine labeled with radioactive sulfur. The labeled total sulfur, inorganic sulfate, and chloroform-extractable sulfur-containing compounds in the urines were estimated by radioactivity measurements. Fifteen % of the sulfur was recovered in the urine 24 hours after injection of 10 mg. of L-cystine and was chiefly in the form of inorganic sulfate. When 150 mg. of bromobenzene were administered simultaneously, the amount of labeled total sulfur excreted appeared in the urine as chloroform-extractable sulfur at the expense of inorganic sulfate. The radioactive sulfur in this fraction was incorporated into bromophenylmercapturic acid since, following the addition of unlabeled bromophenylmercapturic acid to the urine, radioactive bromophenylmercapturic acid was recovered. Twenty % of S^{35} administered as DL-methionine was found in the urine in the form of inorganic sulfate after 24 hours. The administration of bromobenzene caused an increase in the total excretion of the injected S^{35} to 60% without appreciable change in the inorganic sulfate. The increase in excreted S^{35} was not accounted for by the mercapturic acid found but by other organic sulfur compounds not extractable by chloroform. The studies will be extended and correlated with alterations in sulfur metabolism produced by carcinogenic hydrocarbons.

Study of the carbohydrate-regulating hormone of hog adrenal extract. WILLIAM J. HAINES (by invitation), RICHARD H. JOHNSON (by invitation), MARI P. BRUNNER (by invitation), MARVIN L. PABST (by invitation), and MARVIN H. KUIZENGA. *Research Laboratories, The Upjohn Company, Kalamazoo, Mich.* In 1933 Grollman reported that autolysis of beef adrenal tissue caused a significant decrease in the recovery of cortical hormone activity of the survival-growth type. However, subsequent biochemical studies on adrenal extracts, including those resulting in the isolation of 28 pure steroids, appear to have been done with adrenal glands collected by the usual slaughter-house procedures. The large quantities of tissue required in these earlier studies would seem to have prohibited the use of specially-collected glands. More recently we have developed techniques of isolation and identification which allow rather complete characterization of cortical hormone from relatively small amounts of adrenal tissue. These methods have now been applied to the investigation of the carbohydrate-regulating hormone of quick-frozen hog adrenals, in which autolytic changes have been greatly reduced. Comparison of this material, at various stages in its purification, with that obtained routinely from glands collected by by usual procedures, indicates some of the effects of autolysis. The neutral, composite extract from quick-frozen gland contained 120% more bioactivity (liver-glycogen deposition) than that from regular gland. Five hundred mg. (10-kg. gland) of it gave a normal benzene-water distribution after 2 cycles. Long standing in this system caused a 47% loss of activity. Five hundred fifty mg. (11-kg. gland) when chromatographed over $MgSiO_3$, wherein bands were characterized by optical rotation, formaldehyde-forming capacity, and infrared absorption, yielded 70 mg. of 17-hydroxycorticosterone, or 4 times the amount obtained from normal gland. Further characterization of the carbohydrate-regulating hormone are discussed.

Blood changes in acute amino acid deficiencies. W. KNOWLTON HALL, LESTER L. BOWLES (by invitation) and V. P. SYDENSTRICKER (by invitation). *Depts. of Biochemistry, Microscopic Anatomy and Medicine, Univ. of Georgia School of Medicine, Augusta, Ga.* In order to investigate the blood changes which result from amino acid deficiencies, litter mate Wistar-strain rats were placed on diets devoid of one of the indispensable amino acids or on control diets. The deficient diets contained 3 times the minimum requirement of the other indispensable amino acids. Blood was obtained for counts and analyses by sacrifice of the animals and the values obtained with the different groups were compared. Except for some animals which were sacrificed at the beginning of the experimental period, the animals remained on the

diets until the symptoms of the deficiency became extreme. In rats fed a phenyl-alanine-deficient diet there was a reduction in blood volume although the blood counts and plasma protein levels were normal. There was a marked reduction in the number of reticulocytes. In valine deficiency the reduction in blood volume was less marked, there was a slight reduction in plasma protein level and the blood from most of the rats seemed devoid of reticulocytes. In isoleucine deficiency there was a reduction in blood volume, plasma protein levels and the reticulocyte count. These changes may be compared with the extreme hypoproteinemia, reduced blood volume and moderate anemia observed in deficiencies of threonine or protein. There was little difference between control rats fed the mixture containing only the indispensable amino acids and those fed a comparable level of an amino acid mixture similar to casein.

Mode of action of parathormone. PHILIP HANDLER, W. J. A. DEMARIA (by invitation) and D. V. COHN (by invitation). *Depts. of Biochemistry and Pediatrics, Duke Univ. School of Medicine, Durham, N. C.* The intravenous administration of commercial parathyroid extract to dogs consistently resulted in an increased flow of a relatively alkaline urine containing considerably more sodium, bicarbonate and phosphate than the control urines. In some animals this phenomenon was immediately apparent while in others there was a lag period of 15–40 minutes. Studies of inulin and p-aminohippurate clearances revealed that the diuresis occurred simultaneously with a marked rise in renal blood flow and glomerular filtration. These in turn appeared to be secondary to a rise in systemic arterial blood and pulse pressures. The lag period occurred only in those dogs in whom parathormone administration induced a temporary shock-like state with low blood pressure, glomerular filtration and renal plasma flow. In no instance did the parathormone function by actually inhibiting renal tubular reabsorption of sodium, bicarbonate, chloride or phosphate; the altered composition of urine being due solely to the increased glomerular filtration consequent upon the rise in blood pressure and renal plasma flow. Studies are now in progress to determine whether the pressor principle in parathyroid extract is identical with the calcium mobilizing hormone or whether it is an artifact created during the extraction procedure.

Enzymatic hydrolysis of carnosine and related peptides. H. THEO. HANSON (by invitation) and EMIL L. SMITH. *Laboratory for the Study of Hereditary and Metabolic Disorders and the Depts. of Biochemistry and Medicine, Univ. of Utah School of Medicine, Salt Lake City, Utah.* Liver, spleen, and kidney of the rat, and swine kidney are excellent sources of an enzyme, carnosinase, which hydrolyzes carnosine β-alanyl-L-histidine. The swine kidney enzyme has been partially purified. Carnosinase is a metal-protein as indicated by the inhibition produced by cyanide, sulfide, and cysteine. Although both Mn^{++} and Zn^{++} activate the enzyme, it is likely that Zn^{++} is the naturally-occurring metal. This is suggested by the similarity of the Zn^{++}-enzyme and the unactivated preparation with respect to *p*H of optimal activity, poor stability at 40° in absence of substrate, and insensitivity to phosphate and citrate. At 0.05 M carnosine, the hydrolysis follows the kinetics of a zero order reaction, and the rate is proportional to the enzyme concentration over a range of at least 1–12. The optimal action with Mn^{++} is at *p*H 8.0–8.4, with Zn^{++} at 7.8–7.9, and without added metal at 7.4–7.5. The hydrolysis of carnosine cannot be attributed to any previously described protease. In addition to L-carnosine, the enzyme rapidly hydrolyzes both L and D-alanyl-L-histidine, and glycyl-L-histidine while L-α-amino-butyryl-L-histidine, and glycyl-L-histidinamide are hydrolyzed more slowly. D-Carnosine, β-L-aspartyl L-histidine, carbobenzoxy-L-carnosine, and carbobenzoxyglycyl-L-histidinamide are relatively resistant to the enzyme. These data indicate that carnosinase is an exopeptidase of dipeptidase or aminopeptidase specificity.

Application of micro methods to the study of steroid metabolism. J. D. HARDY (by invitation), HILDEGARD WILSON (by invitation) and KONRAD DOBRINER. *Sloan-Kettering Institute for Cancer Research and the Dept. of Physiology, Cornell Univ. Medical College, New York City.* The detailed study of the steroid hormone metabolites in the urine of healthy and diseased persons has been possible in the past with long term collection periods. By the use of infrared spectrometry as a method for the identification of the individual steroids in amounts of 1–3 mg. the period of urine collection was shortened. A further improvement of this specific method of analysis was the development of a microcell for routine use. The cell has a volume of 0.03 ml., and is filled by capillary action. It is designed to be used immediately in front of the slit of a Perkin-Elmer spectrometer so that the entire cross section of the cell is effective in producing absorption. With this cell the individual steroids and their mixtures as obtained by chromatographic procedures can be recognized routinely in amounts of 25 μg. and under favorable circumstances 3 μg. suffice. The methods of separation and chromatographic analysis have been adapted for small amounts and estimation of ketosteroids by the Zimmermann reaction has been modified so that 2 μg. can be determined. This development has made possible a precise qualitative and quantitative procedure for the establishment of steroid excretion patterns in collection

periods of 1–10 days. The details of the methods applied and the results obtained will be discussed.

Toxicity of tetramethyl succinonitrile and the antidotal effects of thiosulphate, nitrite and barbiturates. R. N. HARGER and H. R. HULPIEU (by invitation). *Dept. of Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis, Ind.* Tetramethyl succinonitrile (T.S.N.) and nitrogen are the chief products formed when azo bis-isobutyro nitrile, 'Porofor N' is heated with rubber to make sponge rubber. T.S.N., M.P. 167°, is quite volatile, air saturated with it containing 120 ppm. at 25° and 7820 ppm. at 100°. Animals poisoned with T.S.N. exhibited violent convulsions, with asphyxial death in from 1 minute to 5 hours after the first convulsion. Administered subcutaneously, the LD50 in mg/kg. was about 30 for rats and 23 for guinea pigs. Two rats receiving 49 and 56 mg/kg. per os first convulsed at 5 hours and died several hours later. Intravenously, the LD50 of T.S.N. for rabbits was 20 mg/kg., but 2.5 mg/kg. caused convulsions. Dogs were killed by 20 mg/kg. or more, given subcutaneously. When T.S.N. vapor was inhaled by rats, 60 ppm. killed in 2-3 hours, and 6 ppm. caused death in about 30 hours. Neither artificial respiration, thiosulphate, or thiosulphate followed by nitrite had any effect on the toxicity of T.S.N. Barbiturates administered as needed saved animals receiving up to 50 mg/kg. The use of a quick-acting barbiturate followed by phenobarbital gave the best results obtained. Preliminary experiments indicate that 10 mg/kg. of T.S.N. has a greater analeptic effect than 5 mg/kg. of picrotoxin, when tested in rabbits with severe phenobarbital depression.

Bacterial synthesis in the rat of an unidentified growth-promoting factor. A. M. HARTMAN (by invitation), L. P. DRYDEN (by invitation), and C. A. CARY. *Division of Nutrition and Physiology, Bureau of Dairy Industry, U. S. Dept. of Agriculture.* Although rats require a chemically still-unidentified vitamin, (X), when 35 young were fed an X-deficient diet containing extraordinarily high levels of the 10 B vitamins, methionine and vitamins C, E, and K, 20 rats grew 169 gm. and 15 grew only 87 gm. in 4 weeks. Twelve of the latter increased in growth from 13 gm. per week before to 16, 36, and 32 gm. in successive weeks after being fed a single small dose of feces from the former. Additional slow-growing rats fed sulfasuxidine in the diet did not respond to this feeding of feces. These results suggest very strongly that X may be synthesized by microorganisms which may be induced to thrive in the rat. This synthesis was made possible by the extraordinarily high level of riboflavin, 'inoculations' being ineffective with average intakes of 71 ± S.D. 16 μg/day. Material(s) with X activity as well as with animal protein factor and antipernicious anemia activities is (or are) formed simultaneously microbiologically *in vitro* (Stokstad, *J. Lab. Clin. Med.* 33: 860 1948). Crude preparations of B_{12} and of the 'cow manure' factor are active as X. These results and those of Ott (*J. Biol. Chem.* 174: 1047, 1948) and Bird (*J. Biol. Chem.* 176: 1477 1948) with B_{12} suggest that all of these factors are either very closely related or identical. X occurs in different combinations in different feeds; all are active in the normal rat.

Isotopic studies of the biosynthesis of nucleic acid components. I. Purines and pyrimidines. MILTON R. HEINRICH (by invitation), D. WRIGHT WILSON and SAMUEL GURIN. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* The incorporation of carbon dioxide, acetate, and glycine into the nitrogenous bases of nucleic acids and nucleotides in the rat has been studied. After administering C^{14}-labeled compounds to rats, nucleotides were extracted from the carcasses with acid, hydrolyzed, and adenine isolated. Lipids were removed, nucleic acids extracted with 10% NaCl, and the latter hydrolyzed to individual purines and pyrimidines. Within 7 hours after injecting labeled $NaHCO_3$ into a rat, a very small amount of radioactive carbon was found in the nucleotide adenine and the nucleic acid adenine, guanine, and uracil. Permanganate degradations showed the isotope to be in the non-ureide carbon of guanine, and the ureide carbon of uracil. When an approximately equivalent total amount of isotope in the form of carboxyl-labeled sodium acetate was injected into rats for 5 days, about the same degree of incorporation was observed. No isotope appeared in the ureide or guanidino carbon of guanine. The highest concentration was found in the ureide carbon of uracil, probably due to incorporation of carbon dioxide. Glycine labeled in the carboxyl position was fed to a group of rats for 10 days, using about twice the quantity of isotope given in each of the previous experiments. Incorporation into the purines of nucleic acids and nucleotides was about 20 times that observed with either bicarbonate or acetate. Very little isotope was found in the uracil or thymine. A sample of the nucleic acid guanine was hydrolyzed with concentrated HCl to give glycine in which the carboxyl group is derived from carbon 4 of guanine. This glycine carboxyl, obtained with ninhydrin, contained essentially all the isotope incorporated into the guanine.

Cytochrome *c* reductase (*DPN*). LEON A. HEPPEL (introduced by B. L. HORECKER). *Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* The reduction of cytochrome *c* by reduced diphosphopyridine nucleotide (DPN) has been observed in freshly prepared pig heart suspensions (Lockhart and Potter, *J. Biol. Chem.* 137: 1, 1941) and a partially purified preparation of the enzyme has been ob-

tained from baker's yeast (Altschul, Persky and Hogness, *Science* 94: 349, 1941). Lack of information regarding the properties of the cytochrome *c* reductase (DPN) of animal tissues has prompted this investigation. The enzyme could be stored for long periods in hog liver acetone powder, from which it was extracted with 0.06 M phosphate, *p*H 7.4. The turbid extract was incubated at room temperature with crude steapsin. After this treatment a considerable portion of the activity was precipitated between 0.4 and 0.5 saturation with ammonium sulfate and on addition of water a clear solution was obtained. With extracts not treated with steapsin almost all of the activity was precipitated below 0.3 saturation with ammonium sulfate and could not be redissolved. The ammonium sulfate fraction had a *p*H optimum at 6.8 and the enzymatic activity was precipitated below *p*H 5.3. By lyophilization a stable powder was prepared which is being subjected to further purification.

Degradation of polysaccharides by muscle phosphorylase. Shlomo Hestrin (introduced by C. F. Cori). *Dept. of Biological Chemistry, School of Medicine, Washington Univ., St. Louis, Mo.* Branched polysaccharides (glycogen and amylopectin) could only be degraded partially (40 ± 5%) by recrystallized muscle phosphorylase. β-amylase limit dextrins of the branched polysaccharides were not degraded to a significant extent by the recrystallized muscle phosphorylase. Like β-amylase and potato phosphorylase, the phosphorylase of muscle is thus incapable of degrading starch and glycogen chains beyond their branchpoints. The phosphorylase limit dextrin of glycogen differed, however, from the β-amylase limit dextrin of glycogen in respect to priming activity for muscle-phosphorylase. Since the phosphorylase limit dextrin afforded roughly an equivalent of one mole maltose per calculated end group when treated with β-amylase, the outher chains of the phosphorylase limit dextrin are probably three or more glucose units long. The role of this dextrin in the equilibrium mediated by phosphorylase is discussed.

Ataxia and loss of equilibrium in manganese-deficient rats (motion picture). Robert M. Hill, Dorsey E. Holtkamp (by invitation), A. R. Buchanan (by invitation) and Enid K. Rutledge (by invitation). *Depts. of Biochemistry, Anatomy, and Pathology, Univ. of Colorado Medical Center, Denver, Colo.* Shils and McCollum (*J. Nutrition* 26: 1, 1943) reported the development of ataxia and poor equilibrium in rats on a manganese-deficient diet. Symptoms increased in severity to the 18th day of life when the rats either died or 'recovered to a great extent.' We have seen these symptoms in the 2nd, 3rd, and 4th generations of rats on a very low manganese intake, about 0.006 mg/rat/day. In animals placed on low manganese diet after maturity no symptoms appeared. Symptom appeared in 5 female rats in the 2nd generation, before 6 months and 3 between 6 and 14 months o age. The latter 3 of these animals raised litters although poorly. Of 3 males and 4 females that were allowed to reach maturity in the 3rd generation 1 male and 2 females developed symptoms before weaning. One of these females that did not show symptoms was bred and successfully raised on of a litter. This one 4th-generation rat, a female, i now 80 days of age and exhibits marked symptoms The symptoms appeared earlier in this rat than in those of the 3rd generation. All 8 females with symptoms showed shoulders leaning to the right with the head rotated to the left. The 1 male showed shoulders leaning to the left with the head rotated to the right. After rotation on a turntable all low-manganese animals showed extreme ataxia and loss of equilibrium. Post-rotational nystagmus was normal in direction but slow and of shorter duration than normal.

A 16-oxygenated steroid from adrenocortical tumor urine. H. Hirschmann and Frieda B. Hirschmann (by invitation). *Dept. of Medicine, Western Reserve Univ., and the Lakeside Hospital, Cleveland, Ohio.* The triacetate (I) ($[\alpha]_D^{26} = -101°$ of a triol $C_{21}H_{34}O_5$ (provisionally designated as Compound A (Hirschmann and Hirschmann, *J. Biol. Chem.* 157: 601, 1945)) isolated from the urine of a boy with an adrenocortical tumor has been reduced catalytically to the triacetate $C_{27}H_{40}O$ (II) (m.p. 177–179°, $[\alpha]_D^{30} = -58°$). The same substance has been prepared from the triacetate (III) of the allopregnanetriol-3,16,20 of the urine of pregnant mares (allopregnanetriol-3(β),16(α),20(β) (IV) (Hirschmann *et al. J. Biol. Chem.* in press)). The 20-monoacetate of IV was tritylated in positions 3 and 16 and the ditrityl ether (m.p. 170–172°) saponified in position 20. The 3(β),16(α)-ditritoxyallopregnanol-20 (β) (m.p. 168–171° was oxidized with chromic acid in acetone to the 3(β),16(α)-ditritoxyallopregnanone-20 (m.p. 269–270°) which was reduced with lithium aluminum hydride, then hydrolyzed with acid and acetylated to yield 2 triacetates which were identified as compounds II and III. Substance II, therefore, is allopregnanetriol-3(β),16(α),20(α) triacetate. In view of the change in molecular rotation accompanying the reduction of I it is most probable that Compound A is Δ^5-pregnenetriol-3(β),16(α),20(α). As this triol was obtained from adrenal tumor urine in a yield of about 6 mg/l. it is derived most likely from compounds formed by adrenal tumor cells. This finding seems to warrant an inquiry into the corticoid activity of suitably substituted 16-oxygenated steroids and into the possible presence of such compounds in the normal adrenal cortex.

Dietary manganese and susceptibility to pneumococcal infection. GEORGE H. HITCHINGS, ELVIRA A. FALCO (by invitation) and MARION B. SHERWOOD (by invitation). *The Wellcome Research Laboratories, Tuckahoe, N. Y.* The chief component of the dietary factor which increases the susceptibility of mice to pneumococcal infection (*Proc. Soc. Exper. Biol. & Med.*, 61: 54, 1946; *Federation Proc.* 6: 261, 1947) has been identified as manganese. By the use of a highly purified diet and a minimally infective dose of the pneumococcus (*Type I* SV1 strain) it can be shown that the susceptibility of mice to infection steadily increases with increasing manganese content of the diet to levels as high as 0.6 mg. Mn/gm. of diet. The effect of manganese in some cases can be partially antagonized by a large excess of dietary cobalt. Similar tests with other types and strains of pneumococci and hemolytic streptococci have resulted in the discovery of several with which the same dietary effect on susceptibility is demonstrable.

Effect of dietary manganese on the growth of white rats. DORSEY E. HOLTKAMP (by invitation) and ROBERT M. HILL. *Dept. of Biochemistry, Univ. of Colorado Medical Center, Colo.* Several laboratories have shown that manganese is an essential dietary factor for growth of young rats, but hitherto no uncomplicated dietary study has been made to determine the optimal manganese intake for this function. In the work reported here, 55 female rats, just before breeding, were divided into 7 groups and put on identical manganese 'free' (6 μg/rat/day) but otherwise adequate basal diets with supplements of manganese in the respective groups added at the following average daily intakes per rat: none, 0.1 mg., 1.0 mg., 5.0 mg., 10.0 mg., 20 mg. and 40 mg. These diets were maintained throughout gestation, lactation and the subsequent growth period of the young. Forty-eight litters were obtained of which 38 (194 young) were raised beyond 18 days of age. In the Mn 'free' group 5 litters were born from the 8 females bred, and 3 litters (16 rats) were raised to weaning age. The other 6 groups showed normal birth records with no significant differences between them. Growth records of the young were started on the second and third days and were continued through the 50th day of life. Growth was better with increased manganese supplements up to 5 mg/mother/day. This intake was optimal. Growth was progressively poorer with the higher supplements of 10, 20 and 40 mg/mother/day. At the 40 mg. level growth was similar to that on the Mn 'free' diet. The advantage of the optimal level of manganese intake over both higher and lower levels becomes more apparent the longer the diets are maintained.

Quantitative paper chromatography of penicillins. I. Use of aqueous solution as developing agent. IRVING R. HOOPER, DAVID L. JOHNSON, AND HAROLD D. TAYLOR (introduced by S. B. BINKLEY). *Research Division, Bristol Laboratories Inc., Syracuse, N. Y.* The commonly known penicillins were separated in paper strip chromatography by development with a sodium citrate solution. 2-Pentenylpenicillin and *n*-pentylpenicillin, moving fastest, were not separated. Benzylpenicillin and *p*-hydroxypenicillin, appearing together, were not completely separated from the 2-pentenylpenicillin and *n*-pentylpenicillin. *n*-Heptylpenicillin moved slowest and was completely separated. Schleicher and Schuell 589 Blue Ribbon filter paper strips, ¼ inch wide were spotted with 0.5 to 2.5 units of penicillin in 0.5 to 5 microliters of solution and then were developed by allowing 40% sodium citrate to flow over the strip in a humidified chamber. After development the strips were placed on *Staphylococcus aureus* inoculated trays which were incubated to demonstrate the position of the penicillins on the strip. The results were photographed. By this procedure benzylpenicillin in mixtures has been determined quantitatively without purification or concentration. The percentages of the penicillins present were determined from the maximum widths of the inhibition zones of two strips with different volumes of a penicillin solution placed on one tray. A statistical analysis was made on the accuracy of the quantitative determination of benzylpenicillin in a series of solutions prepared with known amounts of benzylpenicillin, *n*-pentylpenicillin and *n*-heptylpenicillin. For benzylpenicillin content it was concluded that the observed average for two strips should be within 8.5% of the true value 95 times out of 100 and within 4.2% two times out of three.

Cytochrome *c* reductase (*TPN*) from liver. B. L. HORECKER. *Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* Haas, Horecker and Hogness (*J. Biol. Chem.* 136: 746, 1940) have isolated an enzyme from yeast which catalyzes the reduction of ferricytochrome *c* by reduced triphosphopyridine nucleotide ($TPNH_2$). Cytochrome reductase (TPN) has now been purified from hog liver by extraction of acetone powder with Na_2HPO_4, digestion with trypsin, fractionation between 0.45 and 0.70 saturated $(NH_4)_2SO_4$, fractionation with alcohol at *p*H 5.0, adsorption on $Al(OH)_3$ C γ gel, elution with Na_2HPO_4, adsorption on $Ca_3(PO_4)_2$ gel and elution with Na_2HPO_4. This procedure yields a pale yellow solution which is 170-fold purified compared to the acetone powder extract. The purified enzyme has a specific activity in the reduction of cytochrome *c* with excess $TPNH_2$ which is about one-sixth that of pure cytochrome reductase from yeast. In the acetone powder extracts the bulk of the activity is associated with insoluble particles from which it is liberated by the digestion with trypsin. The activity is found in the mitochondrial fraction

prepared from fresh rabbit liver by the method of Hogeboom, Schneider and Pallade (*J. Biol. Chem.* 172: 619, 1948). The specific activity in these granules is identical with that of the liver acetone powder extracts. With purified preparations no reduction of ferricytochrome *c* occurs with reduced diphosphopyridine nucleotide.

Penicillin and amino acid assimilation in staphylococci. ROLLIN D. HOTCHKISS. *Rockefeller Institute for Medical Research, New York City.* Penicillin is known to cause death of growing, but not of non-growing, bacteria. From the work of Gale it appears that those strains of staphylococci which are susceptible to penicillin are dependent upon pre-formed amino acids for growth. Gale has also shown that staphylococci grown in penicillin are subsequently unable to take up glutamic acid normally. It is difficult to know whether this effect is a cause, or a result, of the primary biochemical damage to these growing cells. An effect upon washed cells could not be demonstrated. The author has reported (*Federation Proc.* 6: 263, 1947) that washed, respiring staphylococci can take up amino acids and couple them into cell protein. In the presence of crystalline penicillin G (50 U/mg. of cells), however, the nitrogen compounds produced are recovered largely or entirely in the suspending medium. This is true although the rates and extents of disappearance of amino groups have always been found to be identical in the presence and absence of penicillin, from a variety of simple and complex mixtures of pure amino acids. Similar small amounts of ammonia, esterified phosphate and other side products are produced in the two cases. Under appropriate conditions, simultaneous utilization of simple amino acids by washed staphylococci leads to the disappearance of uracil from the environment; this disappearance is markedly increased by low concentrations of penicillin. Other pyrimidines or purines are not materially affected. The uracil utilized cannot be recovered by hydrolyzing the bacteria.

Purification, crystallization and properties of circulin, an antibiotic from the bacillus circulans group. STACEY F. HOWELL. *Biochemistry Section, V.D. Research Laboratory, U. S. Public Health Service, Staten Island, N. Y.* Circulin is an antibiotic of polypeptide nature and has been isolated in crystalline form from the culture fluid of Bacillus circulans. It is purified by acidifying the culture fluid to *p*H 2.0, adding acetone to 30% followed by saturation with ammonium sulfate. The circulin transfers to the acetone layer. Part of the acetone is removed by distillation *in vacuo*. The circulin is precipitated from the acetone layer by shaking with chloroform. The circulin is dissolved in water and precipitated with ammonium sulfate. The antibiotic is dissolved in 70% ethyl alcohol and crystallized at reduced temperature. Circulin can be recrystallized from a number of solvents including 30% ethyl alcohol, methyl alcohol, 70% isopropyl alcohol, 80% acetone and molar sodium acetate solution. The form of crystal obtained varies with the solvent. Circulin is very stable. Water solutions or dry preparations can be stored at room temperature without inactivation. Circulin has the following properties: M.P. 235 C., α_{20}^{D} −93.3, isoelectric point in 0.02M citrate buffer is at *p*H 7.0 and absorption bands are at 2520, 2580, and 2640 Å. The elementary analysis and molecular weight of 1050 indicate a molecular formula of $C_{41}H_{55}O_{18}N_9 \cdot SO_4$. Crystalline circulin is soluble in water (0.3%), 0.7M ammonium acetate solution (2.0%), ethylene glycol (5.0%), and 70% ethyl alcohol (1.5%) at 25 C. Although the antibacterial activity of circulin is low, 200,000 *E. coli* U/gm., the spectrum is very wide. Circulin is toxic to animals.

Phosphate turnover of various nucleotides in respiring liver homogenates. J. P. HUMMEL and OLOV LINDBERG (introduced by H. A. MATTILL). *Wenner-Gren's Institute, Univ. of Stockholm, Sweden.* To determine the phosphate turnover in the pyridine and flavin nucleotides, a respiring liver homogenate, with glutamate as substrate, was briefly incubated with P^{32} orthophosphate. After deproteinization with trichloroacetic acid, the filtrate was saturated with ammonium sulfate and the liver acid-soluble nucleotides (flavin and pyridine nucleotides, adenylic acid, and small amounts of ATP) were extracted into phenol. The phenol-extracted material was transferred to water by adding excess ether. The water extracts were analyzed by paper chromatography against the following solvents: butanol-acetic acid, butanol-phenol, pyridine, phenol, and 2,4,6-collidine. Radioactivity on the paper strips was measured directly under a Geiger counter. Location of the nucleotides was established by analysis of extracts from the paper. Excellent separations of the components were obtained with phenol and collidine. Radioactivity was found in a fraction corresponding to ATP, another radioactive fraction was associated with the position of flavin adenine dinucleotide. No radioactivity was found with the adenylic acid, flavin mononucleotide, or cozymase fractions. On the assumption that only one of the two phosphorus atoms of flavin adenine dinucleotide can exchange phosphate, the specific activity of this phosphorus atom was of the same magnitude as that of the easily hydrolyzable P of ATP. This suggests that flavin adenine dinucleotide is intimately associated with aerobic phosphorylation.

Anaerobic phosphorylation due to a coupled oxidation-reduction between α-ketoglutaric acid and α-iminoglutaric acid. F. EDMUND HUNTER, JR. *Washington Univ. Medical School, St. Louis, Mo.* When α-ketoglutarate is added to washed

particles from rat liver tissue in anaerobic experiments, essentially no dismutative reactions occur. If ammonium ions are added along with the α-ketoglutarate a rapid reaction takes place. With bicarbonate buffer evolution of carbon dioxide indicates the formation of acid groups. Since the reaction stops when approximately one molecule of carbon dioxide has been evolved per molecule of ammonium ion added, the ammonium ion must participate stoichiometrically rather than catalytically. If ammonia is in excess, the reaction stops when the α-ketoglutarate concentration has fallen to a very low level. These data suggest the almost exclusive occurrence of the reaction: 2 α-ketoglutarate + NH_3 % succinate + CO_2 + glutamate. This dismutative reaction was observed by Krebs and Cohen (*Biochem. J.* 33: 1895, 1939), but they reported negative results for rat liver. When hexokinase and glucose are added there is considerable phosphorylation of glucose coupled with the oxidation-reduction. The inorganic phosphate removal approaches one molecule per molecule of ammonium ion added, which would be equivalent to one per molecule of α-ketoglutarate oxidized to succinate in the proposed reaction.

Interaction of 9-aminoacridines with proteins and nucleic acids. J. LOGAN IRVIN and ELINOR MOORE IRVIN (by invitation). *Department of Physiological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, Md.* The proton-acceptor species of 2-methoxy-6-chloro-9-(1′-methyl-8′-diethylamino-octylamino)-acridine-(SN-12868) and of quinacrine combine reversibly with bovine plasma albumin and with the proteins of fraction III-1 of bovine plasma. This interaction can be evaluated spectrophotometrically with the equation: $pH = pK_1' + \log (D_1 - D) - \log [(D - D_2) + k_1' [p] (D - D_3)]$ in which pK_1' is the apparent ionization exponent for the group on the aromatic nucleus, k_1' is the equilibrium constant for the binding of the first acridine molecule to the protein, [p] is the molar concentration of unbound protein at equilibrium, and D_1, D_2, D_3, and D are the optical densities, at a selected wavelength, when the acridine is completely in the form of the proton-donor species (D_1), the free proton-acceptor species (D_2), the protein-bound acceptor species (D_3), and an intermediate equilibrium state (D). For SN-12868 versus bovine albumin in the presence of 1M NaCl to minimize uncertainties regarding ionic strength, $\log k_1' = 4.37$ (pH 6.7 to 8.3). Binding of this compound by the mixed proteins of fraction III-1 is about one-half the strength of binding by albumin on the basis of total weight of this fraction, and the data suggest a different type of binding. Strong interaction between a cationic species of SN-12868 and sodium ribonucleate (yeast) or desoxyribonucleate (sperm) has been observed spectrophotometrically in buffered aqueous solutions from pH 6 to 8.6. At pH 6.4 and a concentration of the acridine of 4×10^{-5}M, the mid-point of the equilibrium is attained at a concentration of 0.011 gm. of sodium ribonucleate per 100 ml.

Difference in electrophoretic behavior of sickle cell hemoglobin and normal human hemoglobin. HARVEY A. ITANO and LINUS PAULING (introduced by CARL G. NIEMANN). *Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.* The nature of the phenomenon of sickling of erythrocytes in sickle cell anemia suggests that this disease involves a pathological state of the hemoglobin molecule. The electrophoretic behavior of hemoglobin from individuals with sickle cell anemia and from normal individuals has been studied with the Tiselius apparatus with the collaboration of Dr. S. J. Singer. Ferrohemoglobin and carbonmonoxyhemoglobin from sickle cell blood and from normal blood were studied in phosphate buffers of 0.1 ionic strength at pH values of 5.7, 7.0, and 8.0. A significant difference was found between the electrophoretic mobilities of hemoglobin derived from sickle cell anemia blood and from normal blood in both ferrohemoglobin and carbonmonoxyhemoglobin. The curves of mobility against pH are roughly parallel, the isoelectric point of normal hemoglobin being lower than that of sickle cell hemoglobin for each compound. At pH 7.0 sickle cell carbonmonoxyhemoglobin moves as a positive ion while normal carbonmonoxyhemoglobin moves as a negative ion. The most plausible explanation for the observed differences in mobitility is that there is a difference in the ionizable groups in the two hemoglobins. With use of the titration data of German and Wyman it is possible to calculate the approximate difference in net charge between the two hemoglobins. This calculation indicates that the sickle cell hemoglobin molecule in the pH range studied has between 2 and 4 less net negative charges than the normal hemoglobin molecule.

Mechanism of action of phosphoglucomutase. VENKATARAMAN JAGANNATHAN (by invitation) and JAMES MURRAY LUCK. *Dept. of Chemistry, Stanford Univ., Stanford, Calif.* Phosphoglucomutase was prepared from rabbit muscle by extraction, heating and fractionation with acetone and manganous sulfate. The purified enzyme required Mg^{++}, serum albumin, and sodium sulfite for maximum activity at pH 7.5. It contained nondialysable phosphorus. When the enzyme acted on glucose-1-phosphate labelled with P^{32} the enzyme became radioactive while the specific activity of the ester-P decreased. The observed values for the subsequent radioactivity of enzyme and substrate corresponded with those calculated on the hypothesis that an exchange between enzyme-P and substrate-P took place. The radioac-

tivity of the enzyme could not be removed by dialysis but after reaction with non-labelled glucose-1-phosphate the enzyme lost its radioactivity on dialysis. After inactivation by iodine the radioactivity of the enzyme was not removed by a similar treatment. An exchange between enzyme-P and substrate-P is postulated as an essential step in phosphoglucomutase action.

Interaction between diisopropyl fluorophosphate (DFP) and proteins of brain tissue. BERNARD J. JANDORF and PRISCILLA D. MCNAMARA (by invitation). *Biochemistry Section, Medical Division, Army Chemical Center, Md.* Rabbit brain homogenates were incubated with 10^{-7} to 10^{-9} M DFP solutions. With these concentrations, the inhibition of brain cholinesterase progresses at a slow rate and requires more than 4 hours at 38°C. to become maximal. When DFP containing 15–20 mc P^{32}/gm. was used, the initial rate of uptake of P^{32} by the tissue was far higher than the rate of cholinesterase inactivation; near-maximal binding of P^{32} was attained at a time when more than 50% of the cholinesterase activity was still unimpaired. A small but significant uptake of P^{32} was obtained when a brain homogenate, previously treated with a 100-fold excess of unlabelled DFP was incubated with labelled DFP. When radio-DFP was subjected to mild alkaline hydrolysis, the resulting mixture added to brain homogenates produced no inhibition of cholinesterase activity, and no binding of P^{32} by protein.

Mode of inhibition of chymotrypsin by DFP. II. Elimination of fluorine and introduction of isopropyl. EUGENE F. JENSEN, M-D. FELLOWS NUTTING (by invitation), ROSIE JANG (by invitation) and A. K. BALLS. *Enzyme Research Division, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Albany, Calif.* It has previously been shown by us that the esterase and proteinase activities of crystalline trypsin and chymotrypsin are inhibited by diisopropyl fluorophosphate (DFP). Since both activities were inhibited at the same rate and to the same extent, the activities were considered to be due to the same active groups of the enzymes. Only 1.7 moles of inhibitor per mole of enzyme (using 40,000 as the molecular weight) were needed for complete inhibition of chymotrypsin, whereas chymotrypsinogen was not affected by DFP. After complete inhibition the resulting inert protein was obtained in crystalline form. After treatment of crystalline chymotrypsin with DFP containing P^{32}, analysis of the crystalline inert protein showed that the chymotrypsin had accepted 1.6 moles of P per mole of enzyme. Hence all of the phosphorus of the DFP needed for complete inhibition was introduced into the enzyme. Zeisel analyses showed that the isopropyl moiety was also introduced, but only 2.8 equivalents per mole of crystalline chymotrypsin were found. Whether the difference between the amount of phosphorus and that of isopropyl introduced is real or reflects analytical difficulties is not apparent. Fluorine was shown to be absent by spectrographic analysis. Most probably the inhibition reaction is $2\ DFP + XtH_2 \rightarrow Xt(DP)_2 + 2\ HF$ where XtH_2 represents chymotrypsin containing two peculiar, active hydrogens. It is felt that a complete knowledge of the mode of DFP inhibition may lead to an understanding of the mode of action of certain enzymes.

Lipids of the nervous system during *in vitro* degeneration. A. C. JOHNSON (by invitation), A. R. MCNABB (by invitation) and R. J. ROSSITER. *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.* The changes in the concentration of lipids during the *in vitro* degeneration of cat brain slices and sciatic nerve have been determined after incubation in bicarbonate buffer for periods of time from 1 to 14 days. In brain slices incubated for 14 days there was a great decrease in the concentration of total phospholipid with no change in the concentration of cerebroside or total cholesterol. Of the individual phospholipids, there was a decrease in the concentration of sphingomyelin and cephalin and no significant change in the concentration of lecithin. In sciatic nerve incubated for 8 days there was a significant decrease in the concentration of cephalin and a decrease of borderline significance in the concentration of total phospholipid. There was no significant change in the concentration of cerebroside, free and total cholesterol, sphingomyelin or lecithin. Thus, in confirmation of the work of others, there is a phospholipid-splitting mechanism, possibly enzymatic in nature, in tissue of the nervous system. There are many differences between the degeneration of tissue from the nervous system *in vitro* and the *in vivo* degeneration of peripheral nerve (*Rev. Canad. Biol.* 1949 in press).

Chromatographic separation of bile acids: I. Separation of mixtures of cholic and desoxycholic acids. ARTHUR F. JOHNSON (introduced by ARTHUR H. SMITH). *Dept. of Surgery, College of Medicine, Wayne Univ., Detroit, Mich.* The quantitative separation of milligram quantities of cholic and desoxycholic acids has been accomplished by a chromatographic technique. Hydrated magnesium sulfate (Epsom Salts), in the 40–200 mesh particle size range, is employed as the adsorbent. The adsorbed bile acids decrease the intensity of the fluorescence of the magnesium sulfate making it possible to follow the progress of the chromatogram under ultra-violet light. Empirical sectioning of the completed chromatogram is more convenient for routine determinations. Standard 18-mm. diameter chromatographic tubes are packed with dry adsorbent under suction to give

a column 100 mm. in length. The acids are introduced in benzene solution and development carried out with an additional 100–200 ml. of benzene. After development the column is allowed to dry under suction and divided into four equal portions which are eluted with alcohol. Aliquots of the eluates and of the filtrate are analysed by colorimetric or fluorimetric methods. The cholic acid is found in the eluate from the top quarter of the column and the desoxycholic acid in the bottom quarter and in the filtrate.

Inhibitory action of some antioxidants on rat kidney phosphatase. CARTER D. JOHNSTON (introduced by EDWIN P. LAUG). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* The inhibition of alkaline rat kidney phosphatase by several compounds proposed as antioxidants in food has been studied in an exploratory manner. The enzyme system consisted of a crude kidney extract, glycerol phosphate substrate, barbital buffer (*p*H 9.4) and added Mg ions. Hydroquinone (HQ), nordihydroguaiaretic acid (NDGA), propyl gallate, and thiodipropionic acid inhibit this system 20–25% in concentrations of 0.001 to 0.005M. While 0.005M HQ produces 30% inhibition aerobically, it shows no or very little effect when the experiment is carried out anaerobically in evacuated Thunberg tubes. Quinone, however, inhibits to the same extent under either condition, the degree of inhibition ranging from 80% at 0.005M to 20% at 0.0002M. Quinhydrone lies in an intermediate position. The inhibitory action of HQ thus appears to result from its partial conversion to quinone in the alkaline buffer. Propyl gallate and NDGA show inhibition both in the presence and absence of oxygen. At 0.001M there was a greater inhibition aerobically than anaerobically, but still an appreciable effect in the absence of air (25–40% inhibition). The extension of these studies to related compounds and under a variety of experimental conditions is in progress.

Effects of metabolic inhibitors on membrane potentials. NORMAN R. JOSEPH, ERVIN KAPLAN (by invitation) and C. I. REED. *Univ. of Illinois, Chicago Professional Colleges, Chicago, Ill.* Determinations of the membrane potential across the synovialis of the knee in dogs have been made with various electrolytes and nonelectrolytes. With numerous ions the potentials agree with theoretical values calculated from ionic mobilities. With metabolic inhibitors or activators, however, the potentials are of a greater order of magnitude, and appear at lower concentrations. Inhibitors or activators have been classed under five groups: *1*) cytochrome inhibitors, *2*) heavy metals and other sulphydryl inhibitors, *3*) thiols, *4*) inhibitors or activators of succinic dehydrogenase, and *5*) electron acceptors, i.e., iodine or H_2O_2. In general inhibitors produce positive and activators negative potentials. Sulphide yields approximately 400 millivolts, about twice the effect given by cyanide. From the effects of the reagents on the respiratory enzymes, a theoretical explanation of the sign and magnitude of the potentials can be advanced. Hydrogen transfer systems terminate metabolically at one or another of the cytochromes, which are electron acceptors. The electrons are carried to oxygen via cytochrome oxidase, the component having the highest standard redox potential. The mean difference of potential between this component and the original electron acceptor depends on the potential at which electrons are transferred from hydrogen transport systems. The potential difference depends on facilitation or blocking of hydrogen or electron pathways. Asymmetrical increase of the source potential (blocking electrons at cytochrome b) or decrease of the terminal potential (sulphide, cyanide) result in high positive potentials. Decreasing source or increasing terminal potentials produce negative potentials. Experiments on the gastrocnemius of the rat are in essential agreement.

Acetate oxidation by a washed rabbit kidney cortex preparation. GEORGE KALNITSKY. *Dept. of Biochemistry, State Univ. of Iowa, Iowa City, Ia.* A rabbit kidney cortex, homogenized and washed in several volumes of NaCl-KCl solution and finally suspended in one volume of NaCl-KCl solution, oxidizes acetate practically completely. Acetate is utilized, oxygen is taken up, and carbon dioxide is evolved, according to the following equation: $CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$. The activity of the tissue suspension is proportional to the concentration up to 0.9 ml. of the suspension. Veronal, borate and glycine buffers do not inhibit acetate oxidation by this preparation. The *p*H optimum is in the alkaline range, with the peak at approximately *p*H 8.6. This preparation actively oxidizes acetate, lactate, caproate and succinate. Citrate is oxidized at a slightly lower rate. Pyruvate, glucose and glutamate are not appreciably oxidized. Mg^{++} and inorganic phosphate increase the rate of oxidation of acetate. Mn^{++} cannot replace Mg^{++}. Kidney cortex was found to be the best source of this acetate oxidation system. Under the same conditions, liver oxidized acetate only slightly. Brain, spleen, lung, heart muscle and skeletal muscle exhibited little or no activity on acetate. Fluoroacetate and malonate at 0.005M concentrations almost completely inhibit acetate utilization, oxygen uptake and carbon dioxide evolution.

Acetyl precursor in aromatic amine acetylation in pigeon liver extracts. NATHAN O. KAPLAN and MORRIS SOODAK (introduced by FRITZ LIPMANN). *Biochemical Research Laboratory, Massachusetts General Hospital and Dept. of Biological Chem-*

istry, Harvard Medical School, Boston, Mass. The product of the reaction between ATP and acetate in extracts of E. coli which appears to be an acetyl precursor in pyruvate synthesis (Kaplan and Lipmann) was tested for activity in the enzymatic acetylation of sulfanilamide. A partially purified fraction was found to promote acetylation under conditions in which monoacetyl phosphate and diacetyl phosphate were ineffective. Coenzyme A is necessary for the acetylation with this compound. Compared with ATP plus acetate the yields of acetylated amine were 50 to 90% at 25°; at 37° the yield was 25% only, presumably due to the much faster rate of destruction. The reactivity as acetyl precursor in pigeon liver extract is apparent but less pronounced than in coli extract. This may in part be due to a greater stability of the compound in the latter. Present results suggest however that ordinarily no free acetyl precursor may spill into solution. This is indicated furthermore by experiments on the differential competition for the acetyl donor between the acetyl-trap hydroxylamine and aromatic amines of varied acetyl-affinity. The nature of the primary acetyl donor in pigeon liver and in E. coli extracts will be discussed.

Influence of pteroylglutamic acid on glycine and on porphyrin metabolism. CECILIA K. KEITH (by invitation) and JOHN R. TOTTER. *Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock, Ark.* The effect of pteroylglutamic acid (PGA) on growth and on fecal porphyrin production of weanling albino rats receiving standard purified rations or similar rations with 3 per cent sodium benzoate was investigated. The addition of 0.5 mg. of PGA/100 gm. of purified diet did not influence the growth rate of the animals but elevated the average daily fecal porphyrin excretion from 42 μg. for the control group (12 animals, 28 days) to 50 μg. (12 animals, 28 days) in one experiment and in a second experiment from 68 μg. (8 animals, 24 days) to 94 μg. (8 animals, 24 days). The inclusion of 3% sodium benzoate in the highly purified diets reduced the weekly gain in weight from an average of 21 gm/animal to 5.6 gm/animal. The addition of 0.5 mg. of PGA/100 gm. of the benzoate-containing diet resulted in an increase in growth rate to 15.5 gm/week/animal. The average daily fecal porphyrin excretion by these two groups were respectively 27 μg. (13 animals) and 37 μg. (12 animals) per rat over a 28-day period. The addition of 2% glycine to the benzoate-containing diet prevented the reduction in growth rate and in porphyrin production brought about by the benzoate.

Effect of dietary pteroylglutamic acid on liver D-amino acid oxidase of young rats. BARBARA KELLEY (introduced by PAUL L. DAY). *Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock, Ark.* Weanling male albino rats were given a standard purified diet, with and without pteroylglutamic acid (PGA), for several weeks. The oxygen consumption of the liver homogenates from such animals was measured by the Warburg technic using DL-alanine as the substrate. The inclusion of 5 mg. of PGA per kilo of diet resulted in a decreased enzyme activity. The average oxygen uptakes (μl/gm. wet weight of tissue) for 4 groups of control animals receiving the basal diet and for 4 groups receiving the basal diet with added PGA were respectively: at the end of one hour, 136 and 111; at the end of 2 hours, 262 and 213; and at the end of 3 hours, 705 and 525. The addition of an excess of coenzyme (FAD) to the reaction flasks resulted in an increase in the oxygen uptake of both groups. The amount of coenzyme present appears to be a limiting factor in this reaction. Data on the activity of other flavin-containing enzyme systems will also be presented.

Demonstration of specificity of a simplified method for the determination of total cholesterol. FORREST E. KENDALL, LIESE LEWIS ABELL (by invitation), BETTY B. LEVY (by invitation), J. MURRAY STEELE (by invitation) and BERNARD B. BRODIE. *Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., Depts. of Medicine and Biochemistry, New York Univ. College of Medicine, and New York Univ. and Columbia Research Services, Goldwater Memorial Hospital, New York City.* Although it has been recognized that neither digitonin precipitation nor color development with the Lieberman-Burchard reagent is specific for cholesterol, one or both of these reactions form the basis of all methods for cholesterol determination. Studies on cholesterol metabolism and its relationship to arteriosclerosis made it imperative to know the identity of the substances being measured by these reactions. A simple method which avoids digitonin precipitation has been devised for cholesterol. Serum is treated with alcoholic KOH to saponify cholesterol esters. After dilution with water the cholesterol is extracted into petroleum ether. The cholesterol content of an aliquot of the petroleum ether phase is determined by the Lieberman-Burchard reaction. Accuracy of the method was checked by recovery experiments. Specificity was assayed by application of Craig's countercurrent distribution technique to the material extracted into the petroleum ether. At least 98% of the material which gave color with the Lieberman-Burchard reagent was cholesterol. This degree of specificity pertained to sera of abnormal as well as of normal cholesterol levels. The new procedure gave results on 100 sera that agreed with the values obtained with the Schoenheimer-Sperry method. Indirectly this demonstrates also the specificity of the latter procedure.

Oxidation of higher fatty acids by isolated rat liver mitochondria. EUGENE P. KENNEDY (by invitation) and ALBERT L. LEHNINGER. *Depts. of Biochemistry and Surgery, Univ. of Chicago, Chicago, Ill.* It has been difficult to study the enzymatic oxidation of the higher, physiologically occurring fatty acids by preparations of mitochondria from rat liver due to their extreme insolubility and the fact that the fatty acids are themselves potent inhibitors of the oxidase system. By standardizing techniques of preparing substrate solutions it has been found possible to study the oxidation of the normal saturated acids from C_8 to C_{18}. With increase in length of the carbon chain the tendency to form acetoacetate diminishes greatly while the tendency for complete oxidation to CO_2 increases, as indicated by measurements of R. Q., and acetoacetate formed. Purified normal saturated fatty acids (C_{14}, C_{15}, C_{16}, C_{17}) and oleic acid are oxidized essentially completely to CO_2 at high rates as judged by R. Q. measurements. The oxidation of oleic acid has the same cofactor requirements as octanoic acid. Linoleic, linolenic, and vaccenic acids are also oxidized at high rates by the mitochondria. α-Monopalmitin and the more soluble phosphatidic acid prepared therefrom are readily oxidized. It appears probable that oxidation of esterified forms of higher fatty acids is preceded by enzymatic hydrolysis.

Analysis of protein hydrolysates by group separation and paper chromatography of isotopically labelled derivatives. ALBERT S. KESTON (by invitation), SIDNEY UDENFRIEND (by invitation) and MILTON LEVY. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.* The formation of p-iodophenyl sulfonyl (pipsyl) derivatives of amino acids can be carried out quantitatively (Keston, Udenfriend and Cannan, *J. Am. Chem. Soc.* 68: 1390, 1946). The derivatives are separable by paper chromatography (Keston *et al. J. Am. Chem. Soc.*, 69: 315, 1947). The analytical reagent is labelled with I-131. An indicator reagent labelled with S-35 is used to prepare known pure S-35 pipsyl amino acids. When these are added in known quantities to the I-131 labelled unknowns the estimation of the unknown becomes independent of complete resolution and depends only on the S-35/I-131 ratio in any pure fraction. The possibility of errors in identification and of overlapping chromatographic bands is greatly reduced by preliminary separation of the pipsyl derivatives into groups. This may be accomplished by simple counter current techniques. The most hydrophilic pipsyl derivatives (those of aspartic and glutamic acids, hydroxyproline, serine and threonine) constitute group I and are obtained from the aqueous end of a $CHCl_3$- 0.2N HCl extraction system. Group II which consists of glycine and alanine appear predominately in the organic end of a sym. dichloroethane-0.2 N HCl system. For maximum separation chromatographic development on paper is best done with butanol-isopropanol mixtures for glutamic and aspartic acids (Group Ia), with butanol for hydroxyproline, serine and threonine (Group Ib) and with amyl alcohol for glycine and alanine. In each case the solvent is saturated with aqueous N NH_3 solution before use. The bands on the paper are located by radioautography and separated into strips. Constant ratios of counts with and without a filter in successive strips indicates purity. The corresponding S-35/I-131 values are used in the calculations. Several proteins have been analysed by the technique.

Immunochemical studies of factors modifying interaction of egg albumin and rabbit anti-egg albumin. WALTER J. KLEINSCHMIDT (by invitation) and PAUL D. BOYER. *Division of Biochemistry, Univ. of Minnesota, St. Paul, Minn.* The effect of ionic strength and of *p*H on inhibition of the EA-antiEA reaction and on dissolution of preformed EA-antiEA precipitates has been investigated by quantitative immunochemical procedures. Inhibition of precipitate formation increases with concentration of NaCl up to 1M but decreases with higher concentrations. With KBr the inhibition increases with concentration up to 3M. KBr produces slight and NaCl no dissolution of preformed precipitates. Definite limits of *p*H have been established for the EA-antiEA reaction; complete precipitation occurs between *p*H 6.25 and 8.45. Inhibition of precipitate formation is practically complete at *p*H 4.2 and 9.5, and disappearance of preformed precipitate occurs in buffer solutions of *p*H beyond the optimal range. Knowledge of the amino acid composition of egg albumin and γ-globulin allows approximation of the changes of reactive groups with *p*H. Such calculations indicate that aspartic acid, glutamic acid and lysine play a prominent role in antigen-antibody reactions, while histidine is relatively unimportant. Preliminary experiments have been made on the effect of soluble amino acid derivatives, in particular acetyltyrosine, on the EA-antiEA interaction with the view of further defining the importance of various protein groups in antigen-antibody reactions. Sodium acetyltyrosinate at neutral *p*H shows a marked inhibition at 0.15M and nearly complete inhibition at 0.5M concentration. Approximately 50% dissolution of preformed precipitate is effected by 0.875M. These effects are much greater than those due to ionic strength alone and may be related to the importance of tyrosyl residues in the interaction.

Penicillin-cytochrome complexes. I. M. KLOTZ and W. W. WEBER (by invitation). *Dept. of Chemistry, Northwestern Univ., Evanston, Ill.* Spectrophotometric investigations have been made which give direct evidence of complex formation between cytochrome *c* and penicillins G, K and X, respec-

tively. In the presence of any one of these antibiotics at a concentration of 2×10^{-3} molar, in a phosphate buffer at *p*H 7.0, the spectrum of the heme protein (7×10^{-5} molar) in the ferri state, develops 2 horns at 520 and 550 mμ, on top of the characteristic broad absorption with a peak at 530 mμ. The sample of cytochrome which gave the most striking results was assayed by the method of Rosenthal and Drabkin (*J. Biol. Chem.* 149: 437, 1937) and found to be 97% pure. Experiments with another sample assaying 54% cytochrome *c* gave much less pronounced effects. It seems unlikely, therefore, that the observed effects are due to spectral changes in hemin impurities. All of the penicillins were crystalline samples obtained from the Antibiotics Study Section of the National Institute of Health. Of the penicillins examined, K was the most effective in producing the spectrophotometric change, and hence presumably the most strongly bound in this complex. As in other anion-protein complexes, penicillin can be displaced from its cytochrome complex by the addition of a competing anion such as sodium dodecyl sulfate (2×10^{-3} molar). This displacement by another anion indicates the importance of electrostatic attraction in the formation of the complex with the antibiotic.

Effect of arsenic on the metabolism of selenium. HARLAN L. KLUG (by invitation), DONALD F. PETERSEN (by invitation) and ALVIN L. MOXON. *Chemistry Dept., South Dakota Agricultural Experiment Station, Brookings, S. D.* The alleviation of selenium poisoning by arsenic was first reported from this laboratory in 1938. Since then, the study has been extended to various laboratory and farm animals and the use of arsenic remains the only practical way of counteracting selenium poisoning. Relatively little is known about the mode of action of this selenium-arsenic antagonism although it has been suggested that the selenium might be bound by the arsenic in a non-toxic form in the tissues. Three groups of rats were kept in metabolism cages and given controlled dosages *per os* for 2 ten-day periods. One group received 1 mg/kg. of arsenic (arsenite) daily, the second group received 1.4 mg/kg. selenium (selenite) daily and the third group received 1 mg/kg. arsenic plus 1.4 mg/kg. selenium daily. Urine and feces samples for each period were analyzed and at the end of the 20-day period the rats were sacrificed and various tissues analyzed. The results show that arsenic had little effect on the absorption, excretion or tissue deposition of selenium. The conclusion concerning tissue content is also supported by other data from this laboratory. Metabolism studies indicate that the lungs may be an important pathway of elimination.

Enzymes of liver oxidizing tryptophan to a kynurenine-like compound. W. E. KNOX, KATHLEEN MERO (by invitation), W. I. GROSSMAN (by invitation), and V. H. AUERBACH (by invitation). *Rheumatic Fever Research Institute, Northwestern Medical School, Chicago, Ill.* The metabolic conconversion of tryptophan to kynurenine and ultimately to nicotinic acid is evidence for the existence of a new system of enzymes which act on tryptophan. Such a system has been obtained, from liver only, of several animal species. The soluble enzyme preparation carries out the aerobic oxidation of l-tryptophan in the α-position of the indole ring; d-tryptophan is not oxidized. The product of the reaction is a compound similar to kynurenine, but has not yet been completely identified. The properties of the enzymes and the nature of the transformations do not agree with those reported by Kotake *et al.* for the reactions forming kynurenine. The reaction occurs in air, and the rate is doubled by addition of methylene blue. The product is a diazotizable amine with a free α-amino group. Analyses show the reaction to be: 1 l-tryptophan + 2 0 — 1 amine. No CO_2 is produced. The conversion of tryptophan to kynurenine requires the further removal of one atom of carbon. One atom is found as formic acid upon hydrolysis of the reaction product. Simple fractionation does not separate the preparation into different enzymes. On dialysis the need for two co-factors becomes apparent. These have been identified as Mg^{++} and adenylic acid.

Metabolism of testosterone and androstanediol-3α, 17α by liver and kidney homogenates. CHARLES D. KOCHAKIAN, N. PARENTE (by invitation) and H. V. APOSHIAN (by invitation). *Dept. of Physiology and Vital Economics, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* In previous reports it was shown that testosterone is metabolized by rabbit liver slices (*J. Biol. Chem.* 170: 23, 1947), and homogenate (*Federation Proc.* 7 (1): 42, 1948) to Δ^4-androstenedione-3,17, cis-testosterone and traces of unidentified compounds. Also that rabbit kidney homogenate (*Federation Proc.* 7 (1): 42, 1948) converts testosterone in part to Δ^4-androstenedione-3,17. The homogenates of guinea pig liver and kidney also convert testosterone to Δ^4-androstenedione-3,17 but in smaller yields than rabbit tissues. Furthermore, other as yet unidentified ketosteroids are formed in small amounts. These same homogenates convert androstanediol-3α, 17α to one or more ketosteroids. The metabolites, however, account for less than 5% of the original steroid of which approximately 90% was recovered as crystalline material.

Micro molecular distillation of fatty acids. ALFRED E. KOEHLER and ELSIE HILL (by invitation). *Santa Barbara Cottage Hospital and The Sansum Clinic Research Foundation, Santa Barbara, Calif.* Distillation of the neutral fat fatty acids of the blood from a film at .003 micron Hg. for 30 minutes indicates that some of the fatty acids distill readily at 25°. As the temperature is increased more fatty

acids are distilled but at about 60° the curve flattens markedly with only about 75 per cent distilled. This was at first believed to be due to the presence of higher homologues with low boiling points but it was later found that some pure fatty acids undergo changes at the higher temperature that retards their distillation. In general the fatty acids from caprylic to linoleic distill in proportion to their molecular weights at 30°. The following percentages were found: caprylic 100, capric 75, myristic 60, palmitic 32, oleic 20, linoleic 12, and stearic 4. At higher temperatures (40° to 100°) this relationship no longer holds. Palmitic and stearic acid have conventional types of distillation curves but myristic, lauric, oleic, and linoleic acids do not. In fact, myristic and oleic acids at certain higher temperatures may distill less completely than at lower levels. This phenomena is probably explained on the basis of polymorphism. This may occur in the solid phase below the melting point as in the case of myristic acid or in the acids that are liquid throughout the temperature range as in oleic and linoleic acid.

Adaptive enzymatic conversion of *l*-malate to lactate and CO_2. SEYMOUR KORKES (by invitation), ALICE DEL CAMPILLO (by invitation), and SEVERO OCHOA. *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.* As previously reported (Korkes and Ochoa, *J. Biol. Chem.* 176: 463, 1948) *Lactobacillus arabinosus* can be adapted to convert malate to lactate and CO_2. The isolated enzyme system requires Mn^{++} and catalytic amounts of diphosphopyridine nucleotide (DPN). The reaction appears to be the combined result of the two reactions (1) l-malate + $DPN_{ox} \rightleftharpoons$ pyruvate + CO_2 + DPN_{red} and (2) pyruvate + $DPN_{red} \rightleftarrows$ lactate + DPN_{ox}. The second reaction is catalyzed by lactic dehydrogenase while the first is catalyzed by an enzyme which, except for its DPN-specificity, is identical to the one previously isolated from pigeon liver (Ochoa *et al.*, *J. Biol. Chem.* 174: 979, 1948). A study of extracts of acetone powders from either unadapted or malate-adapted cells shows that: *1*) Approximately equal amounts of lactic dehydrogenase are present in either case; *2*) no 'malate' activity can be extracted from unadapted cells while very high activity is obtained from adapted ones; *3*) simultaneously with the appearance of activity toward malate the extracts become able to catalyze the decarboxylation of oxaloacetate to pyruvate and CO_2; a weak fumarase activity also appears. Adaptation to malate can be obtained in a short time with suspensions of resting cells in Warburg vessels if glucose and small amounts of growth medium are also present. Both a supply of glycolytic energy and of amino acids are essential for the adaptation. The isolation, partial purification, and properties of the enzyme system will be described.

Inorganic pyrophosphate in the enzymatic synthesis of DPN. ARTHUR KORNBERG (introduced by W. H. SEBRELL, JR.). *Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* The purification of an enzyme which catalyzes the reaction: nicotinamide mononucleotide (NMN) + adenosine triphosphate (ATP) $\rightleftharpoons$ diphosphophyridine nucleotide (DPN) + inorganic pyrophosphate (P-P), has been reported recently (*J. Biol. Chem.* 176: 1475, 1948). An autolyzate of dried brewer's yeast was fractionated twice with ammonium sulfate and precipitated at *p*H 4.80. The resulting preparation was free of phosphatase activities and was about 1000 times more active per milligram of protein than the original extract. With 50 micrograms of the purified preparation in 1 cc of incubation mixture, equilibrium was reached in 30 minutes at 38° from either direction starting with approximately 2 micromoles each of NMN and ATP or of DPN and P-P. The equilibrium constant, $K = \frac{(DPN)\,(P\text{-}P)}{(NMN)\,(ATP)}$ is approximately 0.4. These findings indicate a mechanism for the synthesis of DPN and for the origin and function of inorganic pyrophosphate. Current studies with partially purified preparations from brewer's yeast suggest that the synthesis of triphosphopyridine nucleotide involves a direct phosphorylation of DPN by ATP.

Fate of the infecting virus particle. LLOYD M. KOZLOFF (by invitation) and FRANK W. PUTNAM. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.* The origin of virus phosphorus and the fate of the infecting virus particle have been investigated using P^{32} to label various components of the *E. coli*-T_6 bacteriophage system. Previous study in synthetic lactate medium revealed that the medium itself is the ultimate source of 70% of the virus P (Putnam and Kozloff, *Science* 108: 386, 1948). Extension of these experiments to nutrient broth has yielded substantially similar results. In experiments on the fate of the infecting virus particle T_6 bacteriophage was labeled by growth on *E. coli* in broth containing P^{32} (2–3 μc/ml.). Bacteria in isotope free broth were infected with the purified radioactive virus (60,000 cts/min.) under conditions producing a single generation of virus. The phage was purified by concentration in the supercentrifuge followed by differential centrifugation. Prior to infection the radioactivity of the labeled phage was 98% sedimentable at 20,000 *g*. Upon infection 60% of the counts were absorbed by the host bacterium. After lysis 60% of the radioactivity was non-sedimentable in the supercentrifuge (50,000 *g*). The low radioactivity of the progeny could be accounted for as unabsorbed labeled phage used for infection. Disruption of the labeled infecting virus particle is shown by the fact that the cts/min/phage of the supercentrifuge effluent was

60 to 400 times that of the progeny. The data indicate that the integrity of the infecting virus particle is destroyed in the course of reproduction.

Pteridines and xanthine oxidase activity. EDWIN G. KREBS (by invitation) and EARL R. NORRIS. *Dept. of Biochemistry, Univ. of Washington, Seattle, Wash.* An enzyme preparation active in the oxidation of either xanthopterin or xanthine was obtained from whey. Spectrophotometric methods as described by Kalckar were used in studying these oxidations. The 'natural' substrate for the enzyme appeared to be xanthine, which was converted to uric acid at a rate about 25× greater than found in the conversion of xanthopterin to leucopterin. Xanthopterin was found to be an effective inhibitor of xanthine oxidation, the inhibition being of the competitive type. The dissociation constant of xanthine with the enzyme was estimated to be 7×10^{-6}, while that of xanthopterin with the enzyme was around 2×10^{-6}. Pteridines with a substitution in the 7-position, such as xanthopterin-7-carboxylic acid, inhibited xanthopterin oxidation as well as xanthine oxidation. These findings may be of significance in explaining some of the observations of Norris and Majnarich on the effects of pteridines in bone marrow cultures or in rats made anemic on a diet containing sulfathiazole. Xanthopterin in low concentrations or dosages was found to stimulate hematopoiesis, but was inhibited in this action by 7-substituted pteridines. In higher concentrations xanthopterin inhibited blood cell formation. This latter effect might be attributed to its possible role as a structural antagonist in purine reactions such as the oxidation reaction discussed here.

Effect of diet composition on rehabilitation following starvation in albino rats. W. A. KREHL and W. P. MCNULTY, JR. (by invitation). *Yale Nutrition Laboratory, Dept. of Physiological Chemistry, Yale Univ., New Haven, Conn.* Experiments conducted with growing and adult albino rats to determine the effect of diet composition on their ability to rehabilitate following 30% loss of body weight as a result of complete starvation led to the following observations: *1*) The efficiency of rats in regaining their original weight bore little or no significant relationship to the re-fed diets, the composition of which varied in protein content from 10 to 60% and in isocaloric substitution of carbohydrate with fat. *2*) Two of three groups of weanling rats held at constant weight for 5 weeks by chronic inanition were re-fed, respectively, on an optimum artificial ration and on an identical diet, except for the isocaloric substitution of fat for carbohydrate. No significant difference in the rate of gain on the two diets was observed. The third group, placed on a self-selection regimen (after Richter, exhibited a distinctly inferior growth rate; in fact, half the animals failed to select a diet which was satisfactory for growth by any criterion. A control group of weanling rats placed on self-selection from the start either grew poorly or failed to grow at all. *3*) Adult rats placed on dietary self-selection after starvation fell into two groups, *a*) those that regained at a reduced rate and *b*) those completely unable to gain. *4*) Adult rats starved a second time after recovery either on a prepared diet or on self-selection made a more rapid recovery on this second trial. Furthermore, 100% of the animals placed on the self-selection regimen the second time rehabilitated successfully.

Some properties of Chymotrypsinogen B and Chymotrypsin B. VIRGINIA KUBACKI (by invitation), KENNETH D. BROWN (by invitation), and M. LASKOWSKI. *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.* Chymotrypsinogen B and Chymotrypsin B were prepared according to previously described methods (*J. Biol. Chem.* 170: 227, 1947; 173: 99, 1948). For the purpose of comparison Chymotrypsinogen alpha and Chymotrypsin alpha were prepared according to Kunitz and Northrop (*J. Gen. Physiol.* 18: 433, 1934–35). Each protein has been recrystallized a minimum of 4 times. Electrophoresis was carried out in a standard 15 ml. cell using a 1% solution of protein in 0.1 μ buffers at 3°C. Each of the 4 proteins showed a single component, indicating purity of the protein. A 1:1 mixture of Chymotrypsinogen B and Chymotrypsinogen alpha showed two widely separated components in approximately equal amounts. The non-identity of proteins of the B type with proteins of the alpha type was further proved by *p*H mobility curves. The zero mobility point for Chymotrypsin B was found at *p*H 4.7, for Chymotrypsinogen B at *p*H 5.2, for Chymotrypsin alpha at pH 8.3, and for Chymotrypsinogen alpha at *p*H 9.1. Solubility studies using Chymotrypsinogen B were conducted according to Kunitz (*J. Gen. Physiol.* 13: 788, 1930). Before the experiment, the material was equilibrated 5 times with the solvent (0.1M acetate, *p*H 5.0, 0.27 saturation with ammonium sulfate). The slight deviation from the theoretical curve for one component might have been due to the experimental conditions, or the presence of a small amount of contaminant.

Bixin and the rate of autoxidation of methyl linoleate. H. O. KUNKEL and WALTER L. NELSON (introduced by JAMES B. SUMNER). *Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.* The rate of oxidation of methyl linoleate dissolved in triacetin in an oxygen atmosphere was measured in the Warburg manometric apparatus at 25°C. The addition of 130 γ or less of bixin to 50 mg. of linoleate in 2 ml. of solution decreases this rate of oxidation of the linoleate, while at the same time the bixin is rapidly decolorized. The destruction of bixin proceeds as an apparent first order reaction with respect to oxygen uptake as

contrasted to a first order reaction with respect to time. When a larger amount of bixin (325 γ to 50 mg. of linoleate) is added the rate of oxidation is considerably increased, while the bixin is destroyed at the same rate as in the first case. In the presence of light from a mercury lamp, bixin at all concentrations used has been found to produce a more rapid oxidation of the linoleate. This is true even when α-tocopherol has been added to the system at the start. In the presence of α-tocopherol, the bixin is not appreciably destroyed until the end of the induction period, after which its destruction is as rapid as in the experiments first described. Preliminary experiments indicate that carotene acts in a manner similar to that of bixin. Data have also been obtained to show that bixin will enhance the antioxidant action of α-tocopherol.

Certain organic anions and amide and peptide linkages. GRANVIL C. KYKER and SANFORD L. STEELMAN (by invitation). *Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill, N. C.* The rates of hydrolysis at 65°C. of the amide and peptide linkages of certain proteins (egg albumin, serum albumin, gelatin and hemoglobin) by hydrochloric acid in the presence of certain organic anions depend on the concentration of the protein, the anion, and the hydrochloric acid and on the nature of the protein. Most important of all is the ratio of the concentration of the protein to the concentration of the anions. The differences in the hydrolytic effectiveness of the organic anions do not parallel, in all cases, the differences in the affinities of the anions of the acids for proteins as suggested by Steinhardt (*J. Research* NBS 29: 315, 1942). A maximum rate of amide hydrolysis is reached with sodium dodecyl sulfonate and egg albumin. This is not the case with the other proteins studied. Sulfosalicylic acid, which has an 'intermediate' anion affinity for wool protein, inhibits amide hydrolysis of egg albumin. The catalytic effect of organic anions on the peptide hydrolysis is limited to the initial stages of hydrolysis and when the protein is further split, the rate tends to approach that of HCl. For the ones studied, when the protein was approximately 25% hydrolyzed the rate began to closely approach that of the HCl. The mechanism for the catalytic effect is uncertain but may be due to the denaturating action of the anion on the protein and to the combination with it. As the hydrolysis proceeds, there is evidently less tendency of the anion to combine and the rate of hydrolysis approaches that of HCl.

Photoelectric colorimetry of pH. GRANVIL C. KYKER. *Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill, N. C.* Since the advent of photoelectric colorimeters, many procedures for the colorimetric measurement of *p*H have appeared as adaptations to various instruments. Such procedures presume the accurate determination of one color of an indicator in the presence of its other. The colors vary reciprocally with *p*H. The selectivity of filters employed by photelometric instruments to yield monochromatic light is generally inadequate to separate quantitatively the two colors. The effect of one color on the other is compensated by the present method to permit accurate measurements of *p*H. The choice filter is selected for each of the colors of an indicator. The indicator is added to a sample and instrumental readings are taken using each filter. The two readings are expressed as a ratio. The ratio varies with *p*H. The procedure affords certain distinct advantages. A source of error is eliminated since the concentration of indicator has no effect over generous limits. When comparing 8, 10, and 12 drops of bromphenol blue, respectively, in 10 ml. samples of standard buffers (*p*H 3.0–4.6) readings varied greatly with concentration (Klett-Summerson: blue and green filters) while the ratio remained constant with *p*H. Indicators, for which neither color is accurately determinable by an instrument depending on filters, may be acceptable. The method has given excellent results in the teaching laboratory; students encountering it the first time obtain titration curves from 5 ml. 0.1M lactic acid which superimpose smoothly upon the theoretical. Calibration is done in the usual manner.

Studies on the metabolism of radioactive sucrose in the rat. ALVIN R. LAMB and GEORGE O. BURR. *Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii.* Recrystallized sucrose, isolated from a sugarcane leaf after photosynthesis in radioactive CO_2, was given by stomach tube to fully-fed mature rats. Immediately after feeding the 200-mg. dose, the rat was placed in a desiccator cage through which air was passed from a tank at a constant rate of 400 cc/min. The CO_2 in the effluent air was trapped in NaOH solution. By means of a by-pass, samples were taken at intervals in $Ba(OH)_2$, beginning 1 minute after the sucrose was given, and each 10 minutes thereafter, with increasing intervals later. The $BaCO_3$ was filtered rapidly on 20 mm. paper, dried and counted, using an area of 2.65 sq. cm. In one experiment net counts per minute were 18.9 at 11 minutes, 92.8 at 21 minutes, 285.1 at 51 minutes, 116.8 at 225 minutes and 15.3 at 10.5 hours. Sucrose, therefore, has been absorbed and oxidized within 11 minutes of ingestion, by a fully-fed animal. The highest points of the curves of respiratory elimination of radioactive CO_2 were reached in 72–81 minutes. The curves dropped quite rapidly thereafter. Radioactive carbon appeared in the urine within 2 hours. The dried sample registered 223.0 counts/min. Uncontaminated feces registered 28.7 counts/min. The animals were killed after 24 hours. A few typi-

cal counts: muscle glycogen, 43.4/min.; bone marrow, 10.9 to 24.7; brain, 19.3; plasma proteins, 32.5; plasma lipids, 5.8; mesenteric fat, 8.7 to 16.6; total carcass residue fat, 7.1.

A new metabolic regulator in mammalian spermatozoa. HENRY A. LARDY and D. GHOSH (by invitation). *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* Bovine epididymal spermatozoa have comparatively low rates of respiration and aerobic glycolysis and a high rate of anaerobic glycolysis. In contrast, ejaculated spermatozoa separated from seminal fluid by centrifugation have much higher rates of respiration and anaerobic glycolysis and a lower rate of aerobic glycolis. Thus the Pasteur effect exhibited by the epididymal cells has almost completely disappeared in seminal spermatozoa. These metabolic differences result from the liberation of an extremely labile, lipid-soluble substance from a bound form occurring in the epididymal sperm cell. Until the substance has been characterized we shall refer to it as a 'Metabolic Regulator'. Additions of minute quantities of the purified regulator to epididymal spermatozoa immediately stimulates their respiration and anaerobic glycolysis and abolishes the Pasteur effect. An assay for the regulator has been developed based on its ability to stimulate the fermentation of Bakers' yeast. Using this assay the regulator has been concentrated about 2000-fold from bull semen. It is present also in the spermatozoa of the boar, ram, rabbit and man. In low concentrations the regulator stimulates the oxidation of pyruvate + fumarate and of succinate by washed residue of rat kidney homogenate. It inhibits glutamate oxidation. The regulator functions by uncoupling phosphorylation from enzymatic oxidations.

Esterification of phosphate coupled to electron transport between dihydrodiphosphopyridine nucleotide and oxygen. ALBERT L. LEHNINGER and MORRIS E. FRIEDKIN (by invitation). *Depts. of Surgery and Biochemistry, Univ. of Chicago, Chicago, Ill.* Previous work in this laboratory has shown that inorganic phosphate labeled with P^{32} is incorporated into an esterified fraction when dihydrodiphosphopyridine nucleotide ($DPNH_2$) is incubated aerobically with washed rat liver suspension, ATP, Mg^{++}, and cytochrome-c. In order to study this phosphorylation more closely, a system was devised in which $DPNH_2$ was continuously generated by action of β-hydroxybutyric dehydrogenase on β-hydroxybutyrate. The oxidation of the latter by molecular oxygen is not complicated by further oxidation of acetoacetate, which is inert in the system. Oxidation of β-hydroxybutyrate by this system in the presence of ATP, Mg^{++}, catalytic amounts of DPN and cytochrome-c, and inorganic P^{32} causes esterification of the latter. Omission of ATP or Mg^{++} has no effect on the rate of oxidation but abolishes the esterification, which is therefore not obligatory for the oxidation. Aging of the enzyme at 30° causes loss of phosphorylating activity without affecting oxidative activity. The phosphorylation can also be decoupled from oxidation by arsenite, dinitrophenol, Ca^{++}, and other substances. Phosphorylation does not occur if cytochrome-c is replaced by methylene blue, brilliant cresyl blue, or ferricyanide. Adenylic acid has been used as an acceptor to study the efficiency of the phosphorylation in non-isotopic experiments. The maximum P:O ratio observed has been 1.10, indicating that for each pair of electrons passing from $DPNH_2$ to oxygen, at least one molecule of phosphate is esterified.

Mammalian tyrosinase. Action on compounds structurally related to tyrosine and dihydroxyphenylalanine. A. BUNSEN LERNER (by invitation), T. B. FITZPATRICK (by invitation), and W. H. SUMMERSON. *Biochemistry Section, Medical Division, Army Chemical Center, Md.* Study of the ability of tyrosinase preparations from the Harding-Passey mouse melanoma to catalyze the oxidation of a variety of monohydroxy and dihydroxy phenolic compounds reveals that, of the various monohydroxy compounds, only tyrosine and its ethyl ester possess significant substrate activity. Compounds such as phenol, diiodotyrosine, 4-methoxyphenylalanine, and tyramine are relatively or completely inert as substrates, even in the presence of catalytic amounts of added dihydroxyphenylalanine (dopa), which we have shown promotes the oxidation of tyrosine by mammalian tyrosinase (*Federation Proc.* 7: 167, 1948). The 3-amino, 3-fluoro, N-formyl, and N-acetyl derivatives of tyrosine proved to be potent inhibitors of tyrosinase activity. Of the dihydroxy phenolic derivatives, only L-dopa and DL-dopa are significantly effective as substrates, although certain of them (arterenol, catechol, homogentisic acid) resemble dopa in their ability to shorten the induction period in the action of the enzyme on tyrosine, and others (epinephrin, hydroquinone, cobefrin) are rapidly oxidized if dopa oxidation is proceeding simultaneously. The bearing of these findings on the question of substrate specificity of mammalian tyrosinase will be discussed.

Physical stress and liver fat content of the fasted mouse. LOUIS LEVIN. *Dept. of Anatomy, College of Physicians and Surgeons, Columbia Univ., New York City.* The present experiments confirm previous findings of other investigators that administration of crude pituitary extract to fasting mice causes mobilization of fat to the liver. There are good indications that this fat mobilizing effect is mediated by the adrenal cortex. Data will be presented to show that pure adrenocortidotrophin (Armour) is as effective as crude pituitary extract. Certain physical stresses thought to activate

the pituitary-adrenal system have been tested with respect to liver fat mobilization. Some of these (exposure to cold; severe exercise) are very effective in causing increase in liver fat. The fat increase may be detected within one hour after beginning of exposure to the stress and increases in intensity as the exposure period is prolonged. Other stresses (exposure to high temperature or to low barometric pressure), though indicated to be very severe by the inability of many of the animals to survive, do not increase the liver fat content but instead have a tendency to cause a loss of liver fat. Parenteral administration of glucose during exposure to an otherwise effective stress or during treatment with pituitary extract prevents most of the liver fat increase. However, comparison with the liver fat content of glucose injected, non-stressed mice indicates that the glucose treatment does not completely inhibit the fat mobilizing effect. All procedures which cause increase in liver fat also produce a relative and absolute liver enlargement but the increased liver weight cannot all be ascribed to the accumulation of fat.

Investigation of phospholipid composition in minute amounts. CELIA LEVINE (by invitation) and ERWIN CHARGAFF. *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.* The procedures for the chromatographic separation of nitrogenous lipid constituents described recently (Chargaff, *et al.*, *J. Biol. Chem.* 175: 67, 1948) have been extended to the quantitative micro estimation of the bases. Serine and ethanolamine, separated by means of butanol–diethylene glycol–water (4:1:1), were determined in aqueous propanol extracts of the respective adsorbates on filter paper by a modification of the photometric ninhydrin method of Moore and Stein (*J. Biol. Chem.* 176: 367, 1948). Parallel guide strips, developed by means of ninhydrin, indicated the position on the chromatogram of the various amino compounds. Between 2 and 15γ of each component in 0.01 cc of solution served for the determination which had an accuracy of $\pm 3\%$. Choline (25 to 100γ) was estimated with a precision of about 5% in separate chromatograms by the planimetric measurement (Fisher *et al.*, *Nature* 161: 764, 1948) of the molybdenum blue spots produced, as described previously, by the successive treatment of the choline adsorbates with phosphomolybdic acid and stannous chloride. Choline standards were run with each series of determinations. The use of these techniques for the study of the composition of various cellular lipid fractions and for the investigation of novel lipid constituents will be discussed.

Isolation and physicochemical characterization of pituitary follicle-stimulating hormone. CHOH HAO LI. *Institute of Experimental Biology, Univ. of California, Berkeley, Calif.* A method for the isolation of follicle-stimulating hormone (FSH) from sheep pituitaries has recently been described (Li, Simpson and Evans, *Science*, in press). Ca(OH) extract of fresh whole sheep glands was used as the starting material. The extract was next fractionated with $(NH_4)_2SO_4$ and FSH activity concentrated in the fraction 0.50–0.75 saturated $(NH_4)_2SO_4$. This fraction was extracted with 40% ethanol; the extractable material was then precipitated by adding ethanol to 80%. The alcohol precipitate was finally frationated again with $(NH_4)_2SO_4$ at *p*H 4.7; FSH precipitated out in the fraction 0.55–0.70 saturated $(NH_4)_2SO_4$. The hormone thus prepared behaves as a single protein in the ultracentrifuge and in electrophoresis. It contains approximately 1.3% mannose, 0.6% hexosamine, 0.6% tyrptophane and 4.5% tyrosine. Qualitative analysis indicated that the hormone contains cystine but no cysteine. The results of elementary analysis of the preparation were: carbon 44.93%; hydrogen, 6.67%; nitrogen, 15.10%. Electrophoretic analysis of FSH solutions at different *p*H buffers gave the following mobilities: $+4.8 \times 10^{-5}$ at *p*H 4.00, $+2.2 \times 10^{-5}$ at *p*H 4.25, -1.5×10^{-5} at *p*H 4.60, -1.9×10^{-5} at *p*H 4.81, -3.0×10^{-5} at *p*H 5.10, -4.2×10^{-5} at *p*H 5.60, -6.5×10^{-5} at *p*H 7.00. These experiments were conducted at 2°C in buffers of 0.10 ionic strength. From these values, the isoelectric point of FSH is estimated to be approximately at *p*H 4.5. Ultracentrifugal study of one specimen of FSH in a Spinco ultracentrifuge at 165,000 times gravity gave a sedimentation constant, $S_{20°,w}$, to be 4.3 S. Without the values of diffusion constant and partial specific volume, an approximate molecular weight of FSH may be estimated to be 70,000.

Relative size of adrenocorticotropically active peptide fragments. CHOH HAO LI. *Institute of Experimental Biology, Univ. of California, Berkeley, Calif.* In previous studies we found that the adrenocorticotropic hormone (ACTH) isolated from sheep glands preserved its hormonal activity after partial acid or peptic hydrolysis. The active peptide fragments were not precipitated by trichloroacetic acid and were readily dialyzable. We have now found that the average peptide length in the active hydrolysates is approximately 8. In 6 *m* HCl at 120°C. for 24 hours, the hormone hydrolysate contained 6.5% of the total nitrogen as amide nitrogen and 79.4% of the total nitrogen minus the amide nitrogen as the amino nitrogen. These values represent an average of 8 determinations from 4 different ACTH preparations. When the hormone was digested at 37.4°C. to the extent of 48–51% with crude pepsin and the hydrolysate was adrenocorticotropically active, it was found that approximately 10% of the total nitrogen in the hydrolysate was liberated as amino nitrogen, and that no free amino acid was detected by the ninhydrin reaction. From the data summarized in the Table the aver-

Experiment No.	% of Digestion	Total N of Hydrolysate	NH_2-N	N in Peptides	Av. Peptide length
		mM	mM	mM	
83	48	1.04	0.111	0 830	7.5
81	48	1.09	0.099	0.865	8.7
86	50	1.07	0.093	0.850	9.1
87	51	1.07	0.113	0.850	7.5

age peptide length in the hydrolysates was computed and found to vary from 7–9. We are concerned with the isolation of the adrenocorticotropical active peptide(s) in these hydrolysates.

Studies on the biological synthesis of cholesterol from acetic acid. HENRY N. LITTLE (by invitation) and KONRAD BLOCH. *Dept. of Biochemistry and Institute of Radiobiology and Biophysics, Univ. of Chicago, Chicago, Ill.* Studies on the formation of cholesterol in animal tissues carried out with the aid of deuterium have shown that acetic acid is employed in the synthesis of both the isooctyl side chain and the cyclic moiety of the sterol molecule (*J. Biol. Chem.* 145: 625, 1942). Experiments have now been carried out in which cholesterol was synthesized in liver slices from either $CH_3C^{14}OOH$ or $C^{14}H_3COOH$. Degradation of cholesterol obtained from these 2 sources shows that the isotope concentration is higher in the nuclear portion than in the side chain when $CH_3C^{14}OOH$ is the precursor and that the isotope concentration in the side chain at least equals that of the cyclic moiety in cholesterol synthesized from $C^{14}H_3COOH$. Cholesterol formed from acetic acid labeled by C^{13} in the methyl and C^{14} in the carboxyl group contains C^{13} and C^{14} in a ratio of about 1.4 indicating that decarboxylations occur at some stage of the synthetic process. Cholesterol obtained by biosynthesis from labeled acetic acid was converted into cholestane and the latter oxidized with chromic acid to yield the isopropyl group of the side chain in the form of acetone. Isotope analysis of this fraction indicates that the isopropyl group is also derived from acetic acid.

Experiments on the mechanism of coupling between respiration and phosphorylation. W. F. LOOMIS (introduced by Fritz Lipmann). *Biochemical Research Laboratory, Massachusetts General Hospital and Department of Biochemistry, Harvard Medical School, Boston, Mass.* Some aspects of the kidney homogenate technique will be discussed. Preparations, when quick frozen in dry ice, were found to retain practically intact respiration and phosphorylation. Freezing and storage has to occur at around −80°C. At higher temperatures fast deterioration occurs. Phosphorylation coupled anaerobically with ferricyanide reduction was studied and a quotient P/2 ferricyanide of approximately 1 was found. Aerobically a minimum P/O ratio of 2.3 was found in analogous preparations. The already reported inhibition of the coupling by m-dinitrophenol is also observed with p-nitrophenol but not with p-nitrophenol phosphate. Similar activity was found with di- and trihalophenols but none with diiodo-tyrosine or thyroxin. The apparent substitution in the respiration of tissue dispersions of phosphate by nitro or halophenols has been further explored.

Determination of gamma globulin in blood serum. JOSEPH M. LOONEY and MARY O. AMDUR (by invitation). *Research Laboratory, Veterans Administration Hospital, West Roxbury, Mass.* A study of the method proposed by Kunkel (*Proc. Soc. Exper. Biol. & Med.* 66: 217, 1947) for the turbidimetric determination of gamma globulin with zinc sulfate indicated that the turbidity varied with the albumin concentration. When albumin was added to a 2.0% solution of γ-globulin the turbidity readings obtained were 5.0, 14.0, 10.8 and 8.5 U, at albumin concentrations of 0.0, 1.0, 3.0 and 8.0% respectively. Irrespective of the γ-globulin concentration on the maximum turbidity was obtained at an albumin concentration of approximately 1.0%. By adding a solution of zinc sulfate to a buffered serum dilution in the presence of gum ghatti a stable opalescent suspensoid, which was proportional to the γ-globulin concentration, was obtained. For the determination, 0.3 ml. of serum was mixed with 4.0 ml. of barbital buffer *p*H 7.5 (187 mg. barbital and 140 mg. Na-barbital/l.) and 2.5 ml. of 2% gum ghatti, and then 1.0 ml. of $ZnSO_4 \cdot 7H_2O$ (192 mg/l.) was added. After 30 minutes the turbidity was read at 650 mμ in a Coleman Jr. spectrophotometer against a blank containing saline in place of the serum. The concentration was determined from a standard curve prepared from pure γ-globulin. The turbidity produced by a given amount of γ-globulin remained constant when the albumin concentration was varied from 0.0–8.0. Recoveries of γ-globulin added to sera were made in a series of 27 experiments. The mean value of the γ-globulin as calculated was 2.20 gm/100 ml., and that found was 2.15 gm/100 ml. The standard deviation of the individual readings was 0.15 gm. The difference between values exceeded 0.20 gm. in only 3 cases. The mean value of γ-globulin found in 32 patients with normal serum proteins was 0.89 gm/100 ml.

Comparative study of growth factors for some lactic acid bacteria. CARL M. LYMAN and J. M. PRESCOTT (by invitation). *Dept. of Biochemistry and Nutrition, Agricultural and Mechanical College of Texas, College Station, Texas.* By means of electrolysis of a dilute solution of liver extract prepared for the treatment of pernicious anemia (reticulogen), the growth factors required by *Leuconostoc citrovorum* 8081 and *Lactobacillus leichmannii* 4797 were separated. The factor for *Leuconostoc citrovorum* migrated toward the positive electrode indicating the acidic nature of the sub-

stance. The factor for *Lactobacillus leichmannii* was found in the electrolysis cells nearest to the negative electrode. Separation of the factor for *Lactobacillus leichmannii* from the other factor was also accomplished by treatment of a dilute liver extract solution with the anion exchange resin IRA 400. The factor for *Leuconostoc citrovorum* was quantitatively retained on the column while almost 100% of the other factor passed through. A striking difference in the stability of the 2 factors toward heating in dilute alkaline solution was found. On autocalving with 0.05 N NaOH at 15 pounds pressure for 30 minutes, the factor for *leichmannii* was completely destroyed while the *citrovorum* factor showed 100% recovery. When *Lactobacillus leichmannii* 313 was used as the test organism, the activity of the various fractions indicated above followed very nearly the same pattern as that obtained with *Lactobacillus leichmannii* 4797. Although the factors for *Leuconostoc citrovorum* and *Lactobacillus leichmannii* appear to be distinctly different compounds, they are very probably related. In a comparative test on the activity of enzymatic digests of various animal tissues, liver and spleen gave the highest values and muscle tissue gave the lowest values with both organisms. The presence of thymidine may have influenced these results.

D-serine inhibition of *E. coli* and its reversal by other amino acids and by mutation. WERNER K. MAAS (by invitation) and BERNARD D. DAVIS. *U. S. Public Health Service, Tuberculosis Research Laboratory, Cornell Univ. Medical College, New York City, and the Naval Medical Research Institute, Bethesda, Md.* D-serine produces a nephrotoxic effect in the rat (Artom *et al., Proc. Soc. Exper. Biol. & Med.* 60: 284, 1945). Animals can be protected against the action of D-serine by several amino acids (Wachstein, *Arch. Path.* 43: 515, 1947). We have found that growth of normal *E. coli* is delayed considerably by concentrations of D-serine as low as 5 γ/ml. In the presence of D-serine the bacteria undergo a few divisions at normal rate. Further multiplication is then retarded for a period whose length depends upon the concentration of D-serine, but eventually the organism overcomes the inhibition. This adaptation is due not to the formation of an adaptive enzyme, but rather to an adjustment of the environment. Of 11 other DL-amino acids tested at 200 γ/ml., none proved to be inhibitory. L-aspartic acid, although not inhibitory by itself, greatly enhances D-serine activity. Complete antagonism is provided by DL-alanine and glycine. Most other amino acids, even in high concentration, produce only partial reversal of the inhibition. L-cysteine, L-cystine, L-hydroxyproline and, curiously, L-serine have no effect. Spontaneous mutants which are resistant to D-serine inhibition occur with relatively high frequency. The mutation rate is greatly increased by ultraviolet irradiation. Resistant colonies when grown on D-serine medium together with sensitive bacteria produce a zone of satellite growth of the sensitive bacteria. The simple structure of D-serine permits this phenomenon to serve as a model for studying the mechanism of drug resistance.

Evaluation of six standard proteins by growth of young dogs. DOROTHEA MABEE (by invitation) and AGNES FAY MORGAN. *Laboratory of Home Economics, Univ. of California, Berkeley, Calif.* This experiment was part of a cooperative effort made by several laboratories to standardize methods of assessing the nutritive value of proteins. Either 2 or 3 comparable young dogs from our purebred cocker spaniel colony were used for each diet. Growth and intake were observed for 120 days, and blood composition and N retention regularly determined. Raw egg yolk was used as the control diet. The proteins were fed at the 18% level except for the egg yolk and whole egg powder which were fed at the 12% level and the gluten and dried egg albumin at both 18 and 40% levels. The apparent absorbabilities were gluten 96, casein 93, casein plus 1.0% methionine 91, beef 89, peanut 88, egg yolk 87, whole egg powder 82, heated (15 minutes at 140°) casein plus 0.6% lysine 78, heated casein 76, heated egg powder 61, egg albumin 45. If the gain per gm. protein eaten for raw egg yolk be taken as 100, that of whole egg powder was 77, beef muscle 73, casein 71, heated casein plus lysine 67, heated casein 65, heated egg powder 61, casein plus methionine 55, peanut flour 41, gluten 28 (18% level) and 34 (40% level), dried egg albumin 25 (18% level) and 9 (40% level). The dried egg albumin contained an active antitryptic agent and the gluten produced running fits. The egg albumin was well utilized by rats showing no evidence of antitryptic activity.

Determination of hemochromogen in serum. WILLIAM M. MCCORD (introduced by J. ROY DOTY). *Dept. of Chemistry, Medical College of South Carolina, Charleston, S. C.* Slight hemolysis of red cells interferes with the determination of hemochromogen in serums. By determination of 'oxyhemoglobin hemin' and 'total hemin' the serum hemochromogen may be calculated by difference. By determining the optical density of a sample of serum, with a Beckman Spectrophotometer, at 576 mμ and at 588 mμ, using water as the blank, the concentration of the hemin, representing the oxyhemoglobin present, may be calculated by using the formula: $\text{Hemin}_{(oxy)} = (\text{O.D.}_{576} - \text{O.D.}_{588}) \times 6.86$ mgs/100 ml. Total hemin is determined by adding 8.5 ml. of 10% ammonium hydroxide and 0.5 ml. pyridine to 1 ml. of serum. Five ml. of the mixture is used as a blank; to 3 ml. of the remainder, in the absorption cell, is added 0.1 ml. of freshly prepared

20% sodium hydrosulphite ($Na_2S_2O_4$). The optical density is read at 555 mμ. The total hemin concentration is calculated by using the formula: $Hemin_{(total)} = O.D._{555} \times 34.72$ mgs/100 ml. Serum hemochromogen, in terms of hemin, is then calculated by difference. Standardization is accomplished by the use of crystalline hemin. Sensitivity of the method is of the order of 1 mg. hemin per 100 ml. with an accuracy of about 5%. Bilirubin does not interfere.

Stability and stabilization of testicular hyaluronidase. D. Roy McCullagh, Florence Valentine (by invitation), and Sibylle Tolksdorf (by invitation). *Dept. of Biochemistry, Schering Corporation, Bloomfield, N. J.* The stability of hyaluronidase which has been dried from the frozen state has been investigated. It has been found to decrease as the purity increases. Bulk enzyme assaying 150 Turbidity Reducing Units per mg. does not measurably decrease in potency on standing at room temperature for several months. 500 TRU/mg. material has a half life of approximately 3 months. Hyaluronidase can be sterilized by filtration without destruction. When it is packaged in vials, losses are occasionally observed during drying. The lability is greater when the amount of enzyme inthe vial is very small. The enzyme in vials is stable for several months at 5°C., but at room temperature it decreases considerably in potency in 2–3 months. At 60°C. the aqueous solution is almost immediately destroyed. In the dry state at 60°C. the half life may vary from a few hours to a few days according to the amount of enzyme in the vial. It has been found that the addition of small quantities of gelatin will greatly increase the stability of hyaluronidase. However, when gelatin is included in the solution to be dried, the final product is not readily soluble. Therefore, a polypeptide derived from gelatin has been used as a stabilizer. When this material is used the potency decreases only 15–20% in 7 weeks at 60°C. The product is very soluble in water. Various bacteriostatic agents have been used in conjunction with hyaluronidase and do not appear to destroy the activity.

Further studies on enzymatic benzoylations. R. W. McGilvery (by invitation) and P. P. Cohen. *Laboratory of Physiological Chemistry, Univ. of Wisconsin, Madison, Wis.* The study of enzymatic benzoylations has been extended to the conjugation of ornithine by the fowl. The washed residue of chicken tissue homogenates couples p-aminobenzoic acid (PAB) with N^{δ}-benzoyl-L-ornithine to form *p*-amino-L-ornithuric acid. The product has been isolated as the *p*-(*p*-hydroxyphenyl) azo derivative. N^{α}-benzoyl-L-ornithine also reacts with PAB, presumably forming *p'*-amino-L-ornithuric acid, but this reaction has not been studied in detail. Adenosine triphosphate (ATP) is required for the synthesis. Other properties of the systems will be reported.

Growth stimulatory factors for the malarial parasite, Plasmodium knowlesi. Ralph W. McKee and Quentin M. Geiman (by invitation). *Depts. of Biological Chemistry, and of Comparative Pathology and Tropical Medicine, Harvard Medical School, Boston, Mass.* Submaximal growth and multiplication of *Plasmodium knowlesi* are obtained when a nutrient medium of known composition (*J. Exper. Med.*, 84: 607, 1946), similating plasma, is used. The addition of at least 2, apparently different, factors stimulate *in vitro* growth and multiplication of the malarial parasities. One factor is a dialyzable component (or components) present in horse, monkey and human plasmas. In a washed erythrocyte culture system, to which are added the nutrient medium and purified serum albumin or globulin, growth and multiplication are retarded. The addition of either plasma or the dialyzable component restores the activity. This growth stimulatory substance is organic and appears to be quite heat stable as deproteinization with heat at *p*H 5 does not destroy the activity. The other active material is a non-dialyzable substance (or substances) present in 15 unit antipernicious anemia concentrate of liver. The liver concentrate (0.005 ml. per culture) increases the rate of growth and the numbers of parasites to as much as 150% of that obtained when plasma alone is present. The addition of folic acid or of additional p-aminobenzoic acid appear to have no stimulatory action. Vitamin B_{12} remains to be tested. Further chemical and isolation studies are under way on these factors.

Rate and mechanism of the Liebermann-Burchard reaction on various derivatives of cholesterol. Herbert McKennis, Jr., Joseph Y. Thomas (by invitation) and J. C. Forbes. *Dept. of Biochemistry, Medical College of Virginia, Richmond, Va., Dept. of Physiological Chemistry, The Johns Hopkins Univ. School of Medicine, Baltimore, Md. and Laboratory for Surgical Research, Medical College of Virginia, Richmond, Va.* During the course of our investigations of the effect of various derivatives of cholesterol on growth it became desirable to know the comparative behavior of these substances in the Liebermann-Burchard reaction. The results of rate studies at bath temperatures of 10° and 22° appear to indicate a process which is kinetically of the first order. In view of the recent isolation of dicholesteryl ether from the spinal cords of cattle (Silberman, N., and Silberman-Martyncewa, S., *J. Biol. Chem.* 159: 603, 1945) it was particularly interesting to study this ether. On a molar basis the amount of color produced was essentially twice that from cholesterol itself. This finding gives indirect support to Yoder's conception of cholesteryl ether cleavage by a cholesterol-cholesterilene route. It further opens to question the view (Nath, M. C., Chakravorty, M. K., and Chowdhury, S. R., *Nature* 157: 104, 1946) that

dicholesteryl ether is not an intermediate in the production of the Liebermann-Burchard color.

Formation of formaldehyde in the biological oxidation of the methyl group of sarcosine. COSMO G. MACKENZIE and VINCENT DU VIGNEAUD. *Dept. of Biochemistry, Cornell Univ. Medical College, New York City.* In the course of studies on the biological oxidation of the methyl group in S- and N-methyl compounds, radioformaldehyde has been obtained as an oxidation product of sarcosine labeled in the methyl group with C^{14}. The formaldehyde has been isolated as the dimedon derivative from the trichloracetic acid filtrate of rat liver homogenates incubated with the labeled sarcosine. This derivative of formaldehyde has also been isolated by direct distillation of the incubation mixture from 0.1 M H_3PO_4 into aqueous dimedon. Free formaldehyde is formed in the course of the oxidation of the methyl group as can be shown by blowing it out of the incubated homogenate-sarcosine mixture with a stream of N_2 at 37° and trapping it in a solution of dimedon. The formation of free formaldehyde from the methyl group of radiosarcosine by respiring liver slices may similarly be demonstrated by drawing a stream of O_2 through the reaction vessel and into a dimedon scrubber. A positive color test for formaldehyde in liver homogenates incubated with sarcosine has been reported by Handler, Bernheim and Klein (*J. Biol. Chem.* 138:211, 1941). The homogenate described by these authors was employed in the present experiment. Radioactive CO_2 has also been obtained as a product of the oxidation of the tagged methyl group of sarcosine both in liver preparations and in the whole animal.

Specific volume of α- and β-casein and relationship of specific volume to amino acid composition. T. L. MCMEEKIN, M. L. GROVES (by invitation) and N. J. HIPP (by invitation). *Eastern Regional Research Laboratory, Philadelphia, Penna.* The specific volume of a protein is of great importance in determining its molecular weight by sedimentation and diffusion. It has been suggested by Cohn *et al.* (*J. Biol. Chem.* 100: Proc. xxviii, 1933) that the apparent specific volume of a protein is largely determined by the volume increments of the amino acid residues of which it is composed. The fairly complete amino acid analyses of α- and β-casein reported by Gordon *et al.* (*Federation Proc.*, this issue), which show considerable-differences in the composition of these proteins, offer the opportunity of testing the accuracy of the hypothesis that the specific volume of a protein is determined by its amino acid composition. The apparent specific volumes of α- and β-casein, as well as unfractionated casein, have been determined in dilute alkali and in acid solutions. The following data illustrate the agreement of the experimentally determined specific volumes with the specific volumes calculated from the amino acid analyses of Gordon *et al.*

	Apparent Sp. Vol. Calculated from Amino Acid Residues	Apparent Sp. Vol. Determined at 25 C.
α-Casein	0.728	0.724
Unfractionated casein	0.732	0.727
β-Casein	0.744	0.740

Lipids of peripheral nerve during Wallerian degeneration. A. R. MCNABB (by invitation), A. C. JOHNSON (by invitation) and R. J. ROSSITER. *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.* It has previously been shown (*Rev. Canad. Biol.* in press) that when a cat peripheral nerve undergoes Wallerian degeneration there is a steady decrease in the concentration of the myelin lipids, i.e. cerebroside, free cholesterol and sphingomyelin, the substances which we have previously suggested are the principle lipid components of the myelin sheath (*Biochem. J.* 43: 573, 578, 1948), and an increase in the concentration of the ester cholesterol. Further observations on the concentration of neutral fat and total fatty acid make it possible to piece together the changes in the distribution of lipids in a nerve when it undergoes Wallerian degeneration. There is an early increase in water content and a reduction in the contration of neutral fat. After 8 days, corresponding in time to the appearance of macrophages along the course of the degenerating nerve, there is a steady decrease in the myelin lipids, i.e. cerebroside, free cholesterol and sphingomyelin. These substances are presumably slowly hydrolysed, and the products of hydrolysis, e.g. glycerol, fatty acid, choline, galactose, sphingosine and phosphate are removed. There is, in addition, a hydrolysis of cephalin and a slower hydrolysis of lecithin. Some of the fatty acids liberated during hydrolysis presumably combine with free cholesterol to form ester cholesterol while others may be converted into neutral fat. Such a process would explain many of the histochemical observations made on degenerating nerve tissue.

Distribution of radiophosphorus in rabbit tissues after injection of labelled diisopropyl fluorophosphate (DFP). PRISCILLA D. MCNAMARA (by invitation) and BERNARD J. JANDORF. *Biochemistry Section, Medical Division, Army Chemical Center, Maryland.* Sublethal doses of DFP, containing 15–20 mc. of P^{32}/gm., were injected intravenously into rabbits. Upon sacrifice of the animals from 10 minutes to 24 hours after injection, the specific radioactivity (P^{32}/unit tissue weight), in decreasing order, was found to be high in kidney, plasma, lung, and liver, moderate in skeletal muscle, heart, and erythrocytes. The specific activity of cerebral tissue was low even after injection of DFP into the carotid artery. Thus, no correlation was found to exist between the cholinesterase activity of these tissues and their ability to retain P^{32} derived from DFP. The magnitude of specific

activity of rabbit organs depended on the time interval between injection and sacrifice of the animal, while their order of relative activity was independent of this interval. Kidney and liver tissue of animals injected with DFP and sacrificed at intervals showed a decrease in total specific activity with time; however, the proportion of protein-bound to total radioactivity in these organs increased steadily during the same interval. In lung tissue, a maximum value for total and protein-bound P^{32} was attained within 10 minutes after injection; these values decreased only slightly during the subsequent 24-hour period. When DFP was replaced with an equivalent dose of sodium diisopropyl phosphate, little or no P^{32} was retained, 24 hours after injection, in the tissues mentioned.

Micro permanganate acid ashing method for determination of serum iodine. EVELYN B. MAN and DOROTHY A. SIEGFRIED (by invitation). *Yale Univ. School of Medicine, New Haven, Conn.* Protein bound iodine in duplicate 1 cc. aliquots of blood serum is precipitated with solutions of zinc sulfate and sodium hydroxide in micro flasks which fit into 250 cc. centrifuge cups. The centrifuged precipitate, after decantation of supernatant fluid, is washed about 3 times with iodine free water to remove as much inorganic iodine as occurs during Lugol's administration. The precipitate is heated with solutions of potassium permanganate and sulfuric acid. Without transfer from the precipitation flask, the digest is reduced with acid arsenite solution and iodine distilled through a micro condenser into a dilute acid arsenite solution. Iodine in the concentrated distillate is measured by recording, during about 5 minutes, the color change with ceric sulfate in Chaney's constant recording colorimeter. The average of duplicate recoveries of inorganic iodine added to zinc hydroxide blanks after centrifugation but before digestion was 2.2 for 2.0, 4.8 for 5.0, 9.2 for 10.0, and 15.1 for 15.0 gamma % of iodine. Serum hormonal iodine determined by this microprocedure has checked with values obtained by the Riggs, Man macro permanganate acid ashing method. This method requires only 2 cc. of serum for duplicate determinations. Precipitation, digestion and distillation are continued in the same micro flask, without transfer. Different concentrations of acid arsenite solution are used for reduction of the digest, collection of iodine in distillate, and quantitation of iodide by ceric sulfate. Constant records of color change eliminate the problem of exact timing and facilitate recognition of irregularities in color fading produced by contaminating substances in the distillate.

Effect of methyl-bis(beta-chloroethyl)amine on blood constituents. GEORGE H. MANGUN. *Dept. of Laboratories, Henry Ford Hospital, Detroit, Mich.* Hartman and Mangun demonstrated that the nitrogen mustards are capable of inactivating viruses and bacteria in the presence of blood plasma and whole blood, and that a variety of plasma and red blood cell constituents are essentially unaffected or only slightly affected by the sterilizing dosage of Methyl-bis(beta-chloroethyl)amine (HN2) (500 mg/l). An exception is the prothrombin time of citrated plasma. When undiluted citrated plasma is treated with 500 mg/l of HN2 at *pH* 7.0, the apparent prothrombin concentration (Quick's one-stage method) drops progressively over a 3-day period at 4°C. to about 20–30% of its original value. The effect of HN2 has been studied on separated preparations of borine thrombin, prothrombin, fibrinogen and accelerator globulin. When treated with 500 mg/l. of HN2 at *pH* to 7.5 at 4°C. the following changes were noted: Thrombin (0.5 mg/ml. of Seeger's preparation #480904, 17,800 U/mg. of tyrosine) is slowly inactivated, showing about ½ of its original activity at 3 hours and 2–3% at 20 hours. Prothrombin (Seeger's #481025, about 500 U/mg.) is rapidly inactivated in 0.5 mg/ml. saline solution, about 1% of the control activity being present at 2½ hours and less than 0.5% at 20 hours. Accelerator globulin (not purified, treated in 1:10 saline) after 20 hours was about 5% of the control activity. The clotting rate and total fibrinogen (Seeger's #470712, 92% clottable) precipitated by thrombin were unaffected by the sterilizing dosage of HN2.

Enzymatic degradation of thymidine. L. A. MANSON (by invitation) and J. O. LAMPEN. *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo.* In a previous study we obtained both phosphorolysis and arsenolysis of hypoxanthine desoxyriboside by enzyme preparations from calf thymus and rat liver. A desoxypentose-5-phosphate was isolated as a product of phosphorolysis and evidence was obtained for its formation by way of the 1-phosphate ester. (*Abstracts*, Sept. 1948 meeting of the American Chemical Society). The mechanism of the action of nucleosidase on the desoxyriboside appears identical with that demonstrated for the riboside by Kalckar. Deutsch and Laser (*Ztschr. f. physiol. Chem.* 186: 1, 1930) and Klein (*Ztschr. f. physiol. Chem.* 231: 125, 1935), using phosphate and arsenate buffers, showed the existence of a separate pyrimidine nucleosidase. We have studied the degradation of thymidine by preparations from dog bone marrow and calf kidney and have again observed both an arsenolysis and a phosphorolysis. The products of the arsenolysis are free thymine and the free sugar. Free pyrimidines inhibit this reaction. The kinetics of the arsenolysis will be discussed. Thymidine was not degraded by a hemolysate of rabbit erythrocytes or a purine nucleoside phosphorylase preparation from rat liver. The products of the phosphorolysis of thymidine are free thymine and a phosphorylated form of the sugar. The evidence for

the latter is that an uptake of inorganic phosphate occurs and little free sugar is found. Also the addition of hypoxanthine increases the rate of formation of free thymine. The preparations contain purine nucleoside phosphorylase, hence this result is in agreement with the formation of a phosphate ester which is removed by reaction with the hypoxanthine. We have not observed any requirement of a dialyzable cofactor (other than phosphate or arsenate) by the pyrimidine nucleosidase.

Groups involved in the Zimmerman and Kober reactions. H. W. MARLOW (introduced by H. C. TIDWELL). *Dept. of Biochemistry, Southwestern Medical College, Dallas, Tex.* The colored compounds formed by the Zimmerman and the Kober reagents are still unknown; hence it has not been possible to determine the exact groups involved in these tests. In the Zimmerman reaction, it has been postulated that a methylene group adjacent to a carbonyl is necessary for color development. In the Kober reaction the presence of an α-diketone was at one time believed to be responsible for the specific color development. With the synthesis of various 16-substituted steroids, it is now possible to shed further light on these color tests relative to the groups involved. Present data indicate that in the Zimmerman reaction a carbonyl group at C_{17}, with adjacent methylene group at C_{16}, is necessary for the highest color development. Steroids in which a carbonyl group is present at C_{16} with two adjacent methylene groups, however, give no color with the Zimmerman reagent. This finding is not in agreement with the contention that all—CH_2—CO—CH_2-groupings (as in acetone, in ethyl acetoacetate and in cyclopentanone) yield strongly positive Zimmerman reactions. 16,17-di-ketosteroids also give a negative Zimmerman. With the Kober reagent, 16-diketosteroids produce no color. However, 16-keto, 17-hydroxysteroids give an intense Kober reaction. Also 16,17-diketosteroids produce no color with the Kober reagent.

Incorporation of C^{14}-formic acid into nucleic acid fractions of rat and pigeon. WALTON H. MARSH (introduced by HARLAND G. WOOD). *Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* While other workers have shown that labeled formate is incorporated into uric acid and hypoxanthine in pigeons, it has not previously been demonstrated to enter the nucleic acid fractions of birds or mammals or to be oxidized by the bird. Radioactive formate was fed to a pigeon and injected intraperitoneally into rats, the time of sacrifice being 3 days in the former and 14 hours in the latter. The nucleic acids of both the rat and the pigeon were radioactive, although the activity was only a small per cent of that administered. The adenine and the guanine from the pigeon nucleic acids were radioactive. It was also found that the formate which was given to a pigeon at the rate of 0.7 mM/hr., was oxidized to CO_2 on the average of 6.5% for the first 12 hours. The rate of oxidation increased very rapidly throughout this period. This oxidation probably was not entirely due to intestinal bacterial action since pigeon liver homogenates were shown to oxidize formic acid at the rate of 0.2 uM/hr./gm. wet weight of tissue. From the respiratory $C^{14}O_2$, only 5% of the activity in the purines could have come from the incorporation of CO_2 whereas, in the rat, on the basis of the high formate oxidation (W. Sakami, *J. Biol. Chem.* 176: 995, 1948), all the radioactivity of the purines could be accounted for by CO_2 fixation. The location of isotope in the purines may shed light on the mechanism of synthesis.

Determination of fumaric acid in animal tissues by partition chromatography. LAWRENCE M. MARSHALL (by invitation), JAMES M. ORTEN and ARTHUR H. SMITH. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.* A method for examining the fumarate content of tissues has been devised. It employs column partition with silica gel as the mechanical support for the aqueous phase over which passes an amyl alcohol-chloroform mixture, the non-aqueous, mobile phase. The sequence of organic acids liberated by the mobile phase from the silica gel column is directly related to the distribution coefficients of the solutes with reference to the system employed. Citric, malic, lactic, succinic, fumaric, beta-hydroxybutyric, acetic and benzoic acids represent a partial list of acids arranged in inverse order of their release from the column. Glutamic, aspartic, and nicotinic acids, like citric, are delivered after fumaric acid. The effluent acids are titrated with 0.004N sodium hydroxide. When the fumaric acid emitted from the column is collected in successive fractions having geometrically increasing volumes, the concentrations of fumaric acid plotted against the fraction number describes a curve which approximates the normal curve of error. The observed curve agrees closely with a theoretical curve calculated from the binomial law. Recovery studies demonstrated the applicability of the technic to animal tissues. The fumarate concentrations, expressed in mg. %, in several tissues of the fasted adult rat maintained on a stock diet show the following values: brain, 15; kidney, 9.5; liver, 7.8; gastrocnemius muscle, 2.3; and blood, less than 0.3. Preliminary studies indicate the applicability of the foregoing procedure to the determination of other related organic acids in animal tissues.

Protein changes in the developing chick embryo. MARGARET E. MARSHALL (by invitation) and H. F. DEUTSCH. *Dept. of Physiological Chemistry, Univ. of Wisconsin, Madison, Wis.* The proteins of the blood serum and amniotic fluid of chick embryos have been studied as a function of time of incubation. No electrophoretic changes in the

residual egg white proteins are apparent in *p*H 8.6 diethylbarbiturate buffer of ionic strength 0.1. The proteins of the amniotic fluid from the 12–19th day of incubation gave patterns identical with those of the egg white proteins. Analyses of the blood serum of embryos, age 10–19 days, revealed marked changes. On the 13th day of incubation the serum contains 102 mg. % protein nitrogen and 8.3 mg. % lipid phosphorus. A large amount of the protein possesses an electrophoretic mobility higher than that of the serum albumin. These faster migrating proteins carry the lipid phosphorus as evidenced by analyses of electrophoretically isolated material and correlating sedimentation studies. By the 19th day of incubation they are present in much lower concentration. Simultaneously the lipid phosphorus appears with slower migrating proteins. Sedimentation analyses of embryo serum in sodium chloride solutions of varying density shows the presence of at least 3 molecular components.

Metabolism of dehydroisoandrosterone in a woman before and after removal of an adrenocortical tumor. HAROLD L. MASON, JOHN J. SCHNEIDER (by invitation) and EDWIN J. KEPLER (by invitation). *Division of Biochemistry and Division of Medicine, Mayo Clinic, Rochester, Minn.* Dehydroisoandrosterone acetate (150 mg/day for 8 days) was administered to a women with a virilizing adrenocortical tumor. The daily amount of 17-ketosteroids excreted increased from an average of 258 to 274 mg., and the proportion of the β-fraction did not change. After removal of the tumor, administration of the same amount of dehydroisoandrosterone acetate increased the excretion of 17-ketosteroids from 2.8 to 52.3 mg/day (average). The β-fraction increased progressively to 38 and 36 per cent of the total 17-ketosteroids on the 7th and 8th days. Androsterone, dehydroisoandrosterone, etiocholan-3(α)-ol-17-one, 3-chlorodehydroandrosterone, Δ^5-androstene-3(β),17(α)-diol, pregnane-3(α), 20(α)-diol and a new alcohol, $C_{21}H_{34}O$, were isolated from the urine during a control period, and during the first period of administration of dehydroisoandrosterone acetate. The quantitative relations of these compounds were essentially the same in the 2 periods. During the period after operation, androsterone, dehydroisoandrosterone, etiocholan-3(α)-ol-17-one and pregnanediol were isolated. It was expected that administration of an amount of dehydroisoandrosterone comparable to the amount being excreted would affect the relations of the various urinary steroids, and that perhaps most of the dehydroisoandrosterone would be excreted unchanged. The only effect, however, was a small increase in the total amount of 17-ketosteroids. After operation considerable dehydroisoandrosterone was recovered from the urine, and androsterone and etiocholanolone were isolated in amounts (43 and 32 mg., respectively) which were considerably greater than the total of the endogenous 17-ketosteroids. This last result is in accord with the conclusion that dehydroisoandrosterone normally is converted largely to androsterone and etiocholanolone before excretion.

Manometric determination of methyl alcohol in blood. M. F. MASON. *Parkland Hospital and Southwestern Medical College, Dallas, Texas.* Methyl alcohol, alone or in the presence of ethyl alcohol, in blood may be determined with accuracy sufficient for forensic purposes by the following manometric procedure which is independent of standardized reagents. The distillate obtained from 10cc. or 20cc. of tungstate filtrate in the Harger method for blood alcohol (*J. Lab. Clin. Med.* 20: 746, 1935) is made to 25 cc. Apparatus 'C' described by Van Slyke *et al.* (*J. Biol. Chem.* 141: 627, 1941) for determining carboxyl-CO_2 is modified by introduction of a side-arm equipped with a stop-cock and cup at the shoulder of the tube. Silicone lubricant is used sparingly in the stop-cocks, phosphoric acid as a joint seal. Five cc. of a bichromate-sulfuric acid mixture (e.g. Anstie's reagent) are placed in the tube and the apparatus evacuated (water pump) several times with shaking. Five cc. of distillate and 0.5 cc. of water for washing are admitted via the cup without loss of vacuum, the apparatus shaken, and boiled for 10 minutes. After cooling to about 45°C. the apparatus is connected to the chamber of the Van Slyke-Neill manometric analyzer and the CO_2 evolved is determined as described by Van Slyke *et al.* (*vide supra*). The blank is determined on acidified, boiled water, treated similarly. Methyl alcohol is calculated from the CO_2 determined. Recoveries of added methyl alcohol with or without ethyl alcohol ranged from 97 to 100%. In interpreting results the specificity features of the bichromate oxidation should be kept in mind.

Preliminary communication on the effect of growth hormone on tissue enzymes. JAMES C. MATHIS (introduced by OLIVER H. GAEBLER). *Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit, Mich.* Growth hormone might promote nitrogen storage by reducing amino acid deamination, or by increasing re-utilization of D-amino acids, therefore its effect on D-amino acid oxidase activity was investigated. The oxidase was assayed according to Axelrod *et al.* (*J. Biol. Chem.*, 134: 749, 1940), but a pyrophosphate buffer of *p*H 8.3 was employed. *In vitro* experiments involving five different oxidase sources and various *p*H values showed growth preparations to be without effect. Growth hormone also did not counteract the oxidase inhibitor effect. Experiments on the intact rat, using two strains, several levels of hormone treatment, groups of both sexes, with both *ad libitum* and paired feeding were performed. The data for one representative series

follow: Five 'plateaued' Sprague-Dawley female rats were given 6 mg. hormone intraperitoneally per day for 7 days and 12 mg. on the 8th day, following which they and their pair-fed controls were killed for enzyme assay. Oxidase activities, and standard deviations, are given in cmm. oxygen uptake per organ/hr/100 gm. final body weight. The essentially constant enzyme activity would indicate that no specific activating or deactivating relationship exists between the hormone and the oxidase. The total amount of functioning enzyme in the liver appears roughly equal to that in the kidney. Effects of growth preparations on other enzyme systems will also be discussed.

Organ	Controls	Treated
Kidney	−1170 ±740	−1220 ±170
Liver	−730 ±470	−1100 ±490

Formazyl color reaction as a possible biochemical analytical tool. NORMAN L. MATTHEWS (introduced by EARL R. NORRIS). *Dept. of Pharmacology, Univ. of Washington, Seattle, Wash., Emory Univ. School of Medicine, Emory University, Ga., and Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Although the formazyls were well known in the older literature, apparently no attempt has been made to use the formation of these highly colored substances for analytical purposes. The following classification serves to indicate the possible wide range of application of the formazyls in this respect: I. Substances which yield formazyls by direct reaction with benzene diazonium salts in *a*) mild acid medium (oxalacetic, acetoacetic, and malonic acids) and *b*) only alkaline medium (pyruvic acid, acetaldehyde, acetone, etc.); II. Substances whose phenylhydrazones yield formazyls when reacted with diazonium salts in alkaline medium (aldehydes, aldose sugars, and certain alpha-ketoacids). Formazyls apparently have not been prepared from aldoses, and only formazyl itself could be isolated from the reaction mixture when mannose phenylhydrazone and glucose phenylhydrazone were studied. The advantages of the formazyls as possible analytical agents are that they are easily formed, the sensitivity is great and the colors are characteristic. Disadvantages are that a mixture of formazyls is often formed from one compound, the reaction is not specific, and there is a certain amount of blank color. Quantitative studies have been made with glucose. It was found possible to achieve excellent color development by simply treating with iodine a solution prepared by adding NaOH to a briefly heated mixture of the glucose sample and buffered phenylhydrazine solution. With proper conditions it is possible to minimize the blank color, quantitate the amount of color with the amount of glucose and limit the effect of interfering substances.

Deamination of crystalline egg albumin. PAUL MAURER (by invitation) and MICHAEL HEIDELBERGER. *Depts. of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, New York City.* Water solutions of crystalline egg albumin (Ea) were treated at 0–2°C. in acetate buffer with $NaNO_2$ at *p*H 4.0 for varying periods. The mixtures were neutralized, dialyzed nitrite-free, and were treated with thioglycollic acid at *p*H 7.5 to reduce any -S-S- linkages formed. After dialysis to remove thioglycollate, two fractions were separated at the *p*H of maximum insolubility, 3.9 to 4.0, one soluble, the other insoluble. Both fractions gave homogeneous electrophoretic and diffusion patterns. The soluble fraction was homogeneous in the ultracentrifuge and showed the properties to be expected of an undenatured, partly deaminated Ea. Complete deamination was not achieved under the mild conditions used. In spite of the care taken, the insoluble fraction, which increased with time of exposure to the deaminating solution, was denatured as shown by its insolubility at the isoelectric point and other-physical properties and by its chemical and immunological reactions. Data are presented on the percentage of deamination, viscosity, $[\alpha]_D$, and cross reactivity of each fraction with calibrated anti-Ea and anti-denatured Ea sera.

Depolymerase activities of calf spleen and thymus cathepsin preparations. MARY E. MAVER and ANTOINETTE GRECO (by invitation). *National Cancer Institute, Bethesda, Md.* In a study of the role of cathepsins in nuclear metabolism it was found that these intracellular proteinases from calf thymus and calf spleen hydrolyzed thymus nucleoproteins and rendered the nucleic acid dialyzable. This dipolymerizing function of the cathepsin preparations differed from that of the pancreatic dipolymerases in showing optimal activity at *p*H 4.5 for both the desoxyribosenucleic acid (DNA) and ribosenucleic acid (RNA). In view of the close association of proteolytic activity with the crystalline depolymerases of the pancreas the correlation of proteinase activity with the dipolymerase activity of the cathepsins is of interest. The spleen enzymes which show the highest catheptic activity of any tissue studied show the greatest depolymerase activity. The same activators and inhibitors which affect the catheptic activities of these enzymes also affected their depolymerase activities. The proteinase activity of the calf spleen cathepsin was found previously to be sensitive to the sulfhydryl reagent, *p*-chloromercuribenzoate. The K value of 22×10^{-3} for the first order reaction in the depolymerization of DNA as determined by viscosity measurements was reduced 48% by the addition of 2.5 micromoles

of *p*-chloromercuribenzoate per mg. enzyme. The same reagent decreased the amount of soluble phosphorus liberated from both DNA and RNA. No inorganic phosphorus was liberated by the cathepsins. Cysteine caused a definite increase in the rate of depolymerization of DNA and in the amount of soluble phosphorus liberated.

Hepato-renal factors in circulatory homeostasis. XXI. Quantitative immunochemistry of ferritin and apoferritin. ABRAHAM MAZUR and EPHRAIM SHORR (by invitation). *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* During a study which resulted in the identification of ferritin as the hepatic vasodepressor, VDM, which is concerned with the regulation of the peripheral circulation (*J. Biol. Chem.*, 176: 771, 1948), the immunochemical behavior of ferritin and its iron-free protein moiety, apoferritin, was investigated. Using the quantitative-precipitin technique, it was shown that horse liver and spleen ferritin are identical. Similar immunochemical identity was established for horse ferritin and apoferritin. The iron in the specific precipitates accounted quantitatively for all of the antigen added in the region of excess antibody and up to the end of the equivalence zone. Thus, in these zones, the antibody N in the precipitate may be calculated by subtracting the added antigen N from the total N of the precipitate. Calculation of the molar ratios of rabbit serum antibody ($\overline{M}$ 150,000) to apoferritin ($\overline{M}$ 465,000) gave values corresponding to the formulae, $Antibody_{26}$ Apoferritin in the zone of extreme antibody excess, Ab_{14}Apo at the antibody excess end of the equivalence zone and AB_7Apo at the antigen excess end of the equivalence zone. Inhibition of precipitation was observed with large excess of antigen. By the micro-precipitin technique, using the Folin color reagent to estimate the precipitated protein, 1 to 2 gamma ferritin N could be determined. By means of the complement-fixation test, 0.05 gamma ferritin N was detected. Immunochemical cross reactions were obtained between horse and dog ferritins and to a lesser extent between horse and human ferritins.

Hepato-renal factors in circulatory homeostasis. XXII. Chemical properties of ferritin and apoferritin. ABRAHAM MAZUR and EPHRAIM SHORR (by invitation). *Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.* The identity of ferritin with the hepatic vasodepressor, VDM, which is concerned with the regulation of the peripheral circulation (*J. Biol. Chem.*, 176: 771, 1948) led to a study of some chemical characteristics of ferritin and its iron-free protein moiety, apoferritin. Fractionation with varying concentrations of $(NH_4)_2SO_4$ yielded preparations with widely differing Fe content. The original ferritin contained 1.88 mg. Fe/mg. N. The fraction precipitated at 23% saturated $(NH_4)_2SO_4$ had 2.20 mg. Fe/mg. N, whereas that precipitated between 40–50% saturated $(NH_4)_2SO_4$ had 0.55 mg. Fe/mg. N. All fractions gave typical crystals with $CdSO_4$. The ration of Fe:P in all fractions as constant. A similar ractionation was obtained by centrifuging a ferritin solution at 13,000 rpm. Electrophoresis studies with ferritin and apoferritin yielded identical mobilities at *p*H values from 3.95 to 8.6. An approximate isoelectric point of 4.4 was thus obtained. Amino acid analyses of apoferritin and ferritin yielded identical values for both on the basis of N content. Some amino acid values for apoferritin were (in %): glutamic (17.0), leucine (19.1), arginine (9.0), lysine (7.5), histidine (4.8), phenylalanine (5.9), tyrosine (4.8), valine (4.3), cystine (1.7), methionine (1.8). The total S of apoferritin (0.89%) was accounted for by cystine and methionine. The action of crystalline pepsin and trypsin on ferritin and apoferritin was also studied.

Comparison of the electrophoretic components of human serum at *p*H 4.5 and 8.4. JOHN W. MEHL, FLORENCE GOLDEN (by invitation), and JANE HUMPHREY (by invitation). *Dept. of Biochemistry and Nutrition, University of Southern California School of Medicine, and the Los Angeles County General Hospital, Los Angeles, Calif.* At a *p*H of 4.5, two components with isoelectric points below those of serum albumin may be distinguished. One of these has been identified as a mucoprotein, and has been found to increase in cancer, pneumonia, and a number of other conditions. This component contributes to the α-globulin fraction at *p*H 8.4. The other acid component is also increased in conditions in which the most acid fraction is increased, and may be also a mucoprotein. Three globulin fractions are obtained at *p*H 4.5, one of which can be identified with γ-globulin, and a second with β-globulin. The third component must be made up of part of the α-globulin indicated by electrophoresis at *p*H 8.4. The quantitative relations between the components observed at *p*H 4.5 and *p*H 8.4 will be discussed, and possible applications to the study of pathological sera considered.

Metabolism of 2,4- and 3,5-diketo acids. ALTON MEISTER (introduced by JESSE P. GREENSTEIN). *National Cancer Institute, Bethesda, Md.* The enzymatic hydrolysis of 2,4-diketo acids yielding pyruvic and fatty acids, which was previously reported, may be followed by an ultraviolet absorption technique. Such a procedure was employed in following the metabolism of 3,5-diketohexanoic acid and its δ-lactone. The lactone was enzymatically converted to free 3,5-diketohexanoic acid and the free acid was hydrolyzed to yield aceto-

acetic and acetic acids by homogenates of rat liver and kidney. Lactonase activity was associated with the sedimentable fraction of liver homogenate and had a *p*H optimum at about 6, while the activity toward 2,4- and 3,5-diketo acids remained in the supernatant after high speed centrifugation. The *p*H optimum for hydrolysis of 2,4- and 3,5-diketohexanoic acids was 7.3 to 7.9. Hydrolysis of 2,4- and of 3,5-diketo acids are analogous in that both reactions involve splitting of a C-acyl group. 2,4-diketohexanoic acid was hydrolyzed 11 to 14 times more rapidly than was 3,5-diketohexanoic acid by liver and kidney of several species, by mouse hepatoma, and by partially purified enzyme preparations. Under conditions where n-hexanoic, β-ketohexanoic, and 2,4-hexadienoic acids were oxidized rapidly by a sedimentable fraction of rat liver fortified as described by Lehninger, both 2,4- and 3,5-diketohexanoic acids were metabolized at negligible rates. This suggests that these polyketo acids as such are not intermediates in the oxidation of n-hexanoic acid in this system, but are probably involved in other metabolic pathways.

Synthesis and microbiological testing of some alpha-aminosulfonic acids. JULIUS MENDEL and DONALD W. VISSER (introduced by HARRY J. DEUEL, JR.). *Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles, Calif.* Several alpha-aminosulfonic acids have been prepared which competitively inhibit the naturally-occurring alpha-aminocarboxylic acids. McIlwain has reported considerable overlapping in the growth-inhibiting ability of the aliphatic alpha-aminocarboxylic acids. It was of interest, therefore, to synthesize an aliphatic aminosulfonic acid having no structural analog among the naturally-occurring amino acids. Alpha-aminopropanesulfonic acid and alpha-amino-beta-bromobutanesulfonic acid were synthesized by the addition of ammonium bisulfite to a slight excess of the appropriate aldehyde. The alpha-aminopropanesulfonic acid was antagonistic toward *Lactobacillus casei* at levels from 10^{-2} to 10^{-4} M. Slight reversal of antagonism was observed in the presence of alpha-aminobutyric acid, but no reversal of growth occurred with any of the other amino acids or mixtures of amino acids. Alpha-amino-beta-bromobutanesulfonic acid inhibited the growth of *Lactobacillus casei* completely at 10^{-4} M concentration.

Quantitation of the growth-retarding and anemia-producing effects of hexahomoserine. E. T. MERTZ, R. H. WALTZ, JR., D. C. SHELTON, L. P. DOYLE, and A. L. DELEZ (introduced by F. W. QUACKENBUSH). *Dept. of Agricultural Chemistry and Veterinary Science, Purdue University, Lafayette, Ind.* Young white rats were divided into groups of 6 and maintained for 28 days on a basal diet containing zein, tryptophan, histidine, and 2% DL-lysine·HCl. Each group received, in addition to the basal diet, a different level of DL-hexahomoserine (α-amino, ε-hydroxy caproic acid). When the diet contained 0.08% to 0.33 DL-hexahomoserine, the growth rate was retarded without the simultaneous development of anemia. When the diet contained 1% DL-hexahomoserine, the growth rate was either retarded or reduced to zero, and the animals developed anemia. The rats were autopsied at the end of the experiment, and the autopsy findings will be discussed.

Electrometric method for the determination of red blood cell and plasma cholinesterase activity. HARRY O. MICHEL (introduced by WILLIAM H. SUMMERSON). *Biochemistry Section, Medical Div., Army Chemical Center, Md.* Red blood cell and plasma cholinesterase activity is determined in terms of the change in *p*H produced at 25° by the action of the enzyme on acetylcholine in a standard veronal-phosphate buffer, initially at *p*H 8.00. The *p*H change is measured with the glass electrode, and the reaction time is 1–1½ hours. Separate buffer solutions are used for red cells and plasma; the capacity of the buffers is such that relatively large variation in buffer capacity of added red cells or plasma do not affect results, and the decrease in enzymatic activity with decrease in *p*H is compensated for by an equivalent decrease in buffer capacity over the same range. Small correction factors are provided for non-enzymatic hydrolysis and for slight deviation in rate of *p*H change with *p*H. Only 1 ml. of a 1:50 dilution of plasma or hemolyzed red cells is required for a determination. The method compares favorably with the standard manometric procedure, and is much more suitable for routine use.

Metabolic conversion of the carbon chain of DL-lysine-ε-C^{14} to L-arginine in the dog. LEON L. MILLER. *Dept. of Radiation Biology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* DL-Lysine-ε-C^{14} (2.00 gm., 1.24×10^8 disintegrations/min.) was fed mixed with meat protein to a normal dog. The dog was killed with perfusion 24 hours later. Tissue and blood proteins were hydrolyzed with 20% HCl, and the basic amino acids separated with the ion exchanger Amberlite IRC-50. L-Arginine was isolated as the monoflavianate, after several reprecipitations from

	C^{14} DISINTEGRATION/MINUTE/MILLIMOL $\times 10^{-4}$			
	Arginine monoflavianate	Guanidinium moiety	Ornithine moiety	Glutamic acid
Liver	0.329	0.13	0.19	0.750
Plasma protein	0.323	0.15	0.13	0.607

solution containing added inert L-lysine failed to change its activity. The flavianate was assayed as

such and a portion decomposed with barium hydroxide. The arginine was converted to ornithine, carbon dioxide and ammonia by further treatment with baryta in a sealed tube. Ornithine was isolated as a diparatoluenesulfonyl derivative which was assayed as such. The ornithine moiety contained about 50% of the activity present in the arginine flavianate. The rest of the activity was found in the CO_2 of the guanidine moiety. The activity of glutamic acid isolated from the same tissues is included for comparison. Because the glutamic acid had only about 4 times the activity of the ornithine, it seems more likely that both were derived from a common precursor, rather than that ornithine is derived from glutamic acid through several intermediates postulated by others for such a conversion.

Metabolic conversion of the carbon chain of DL-Lysine-ϵ-C^{14} to L-glutamic and L-aspartic acids in the dog. LEON L. MILLER and WILLIAM F. BALE (by invitation). *Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* DL-Lysine-ϵ-C^{14} (2.00 gm. with 1.24×10^8 disintegrations/min.) was fed with meat protein to a normal dog. When killed 24 hours later, approximately $\frac{1}{3}$ of the activity had appeared in the expired CO_2, $\frac{1}{3}$ in the urine and the rest incorporated in tissue and blood proteins. The proteins were hydrolyzed with 20% HCl, and the dicarboxylic amino acids separated with the ion exchanger Amberlite IR-4. Glutamic acid was isolated as the hydrochloride and aspartic acid as the copper salt. Paper partition chromatography was used routinely to control the separations and as an added criterion of purity of isolated amino acids. No evidence for the occurrence of α-aminoadipic acid was found. The most likely interpretation of the data must postulate more or less direct conversion of the carbon chain of lysine to that of glutamic acid by a hitherto unrecognized mechanism.

Podophyllotoxin on enzymes and tissue metabolism. ZELMA BAKER MILLER, CLARKE DAVISON (by invitation) and PAUL K. SMITH. *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.* Podophyllotoxin, a carcinoclastic agent from podophyllin, was investigated gainst various metabolic systems. Oxygen uptake of rat kidney slices is progressively inhibited by 10^{-3}M podophyllotoxin (74% at 3 hours). Podophyllotixin at 10^{-4}M inhibits to a less degree. The inhibition is greater in the absence of added substrate than in the presence of alanine, pyruvate, succinate and glutamate. Acetate oxidation is inhibited. The oxygen uptake of rat brain and liver slices, and of rat testes is only slightly reduced. The respiration of whole 5-day chick embryo is inhibited in the absense of, but not in the presence of, glucose. Oxygen uptake is stimulated by podophyllotoxin below 10^{-3}M. Anaerobic glycolysis of 5-day chick embryo is inhibited 30-60 % by 10^{-3}M podophyllotoxin; that of rat testes is inhibited 40–75%. Stimulation is sometimes observed at lower concentrations. Rat brain glycolysis is not affected. Carbohydrate synthesis from kidney slices and pyruvate is inhibited 40% by 10^{-3}M podophyllotoxin. Acetate and butyrate, but not glucose, oxidation by rabbit kidney homogenates is markedly inhibited by 10^{-3}M podophyllotoxin. Succinoxidase (pigeon breast), choline and hypoxanthine oxidases (liver), choline esterase (brain), leucine oxidase (*Proteus vulgaris*), adenosine triphosphatase (liver), serum acid and alkaline phosphatase, ribonuclease (spleen), and thymonucleodepolymerase (dog serum) are not affected by this concentration. The viscosity of highly purified thymonucleic acid is not decreased by 30^{-3}M podophyllotoxin.

Prolamines treated with nitrogen trichloride. III. Sulfated zein. FERNANDA MISANI (by invitation, PHILIP WEISS (by invitation), M. G. CORDASCO (by invitation), T. W. FAIR (by invitation), and L. REINER. *Research Laboratories of Wallace & Tiernan Products, Inc., Belleville, N. J.* The NCl_3-treated zein was sulfated according to the procedure of Reitz *et al.* (*J. A. C. S.* 68: 1024). The resulting product contained 1.9% sulfate sulfur and was soluble in water at neutral pH values. It possessed the typical fit producing toxicity of the parent compound. If zein was first sulfated and then treated with NCl_3 a non-toxic product resulted. The NCl_3 consumption by sulfated zein was at least as great as that of the native zein. It appears that sulfation blocked in some manner the reactive group. Sulfamation is hardly responsible for the blocking effect since it has been shown previously that $-NH_2$ groups are not involved in the toxogenic reaction. Esterification of -OH and -SH groups, sulfonation and denaturation through dehydration might have occurred. One of the groups involved in these reactions must also be involved in the production of a toxic residue through reaction with NCl_3.

Glycogen content of the liver of fasted alloxan diabetic rats. YOSHIKAZU MORITA (by invitation) and JAMES M. ORTEN. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.* In the course of investigations on the effect of inorganic and organic phosphate upon the glucose utilization of alloxan diabetic rats, it became necessary to determine the liver glycogen levels in fasting diabetic animals. A survey of the literature showed little information on this question. Male Sprague-Dawley rats, 150–250 gm. in weight were rendered diabetic by injecting alloxan. Liver glycogen and blood sugar levels were determined on 24-hour and 48-hour fasted animals, and the results compared with values found

in normal controls. Twenty rats were used in each group. In the 24-hour fasted animals, the diabetic group averaged 2.15 gm. ± 0.277 (the standard error of the mean) per 100 gm. of liver, while the normals averaged 0.324 ± 0.053 gm. %, a highly significant difference. In the 48-hour fasted group, the diabetic animals showed 0.679 ± 0.090 gm. %, also a highly significant difference. The fasting blood sugar for the 24-hour fasted group of diabetic animals averaged 153 ±22 mg. %, for the 48-hour fasted animals, 90 ± 7 mg. %. The blood sugar values for the control group of rats were within the normal range (55–90 mg. %). Studies on the effect of inorganic phosphate and hexose phosphate on the glycogen content of the liver in the alloxan diabetic rat are now in progress.

Position of the oxygen dissociation curve in cyanotic congenital heart disease. MINERVA MORSE, DONALD E. CASSELS (by invitation) and MELBA HOLDER (by invitation). *Dept. of Pediatrics, Univ. of Chicago, Chicago, Ill.* In spite of decreased arterial oxygen saturation, the arterial oxygen content in cyanotic congenital heart disease is usually as high or higher than normal because of a compensating polycythemia. It is possible, however, that the tissues suffer anoxia due to the low oxygen tension associated with lowered saturation. The position of the oxygen dissociation curve has been examined to determine whether the affinity of hemoglobin for oxygen has been altered by compensatory processes in such individuals. Data were derived from Van Slyke analyses of blood samples equilibrated at 37°C. with gas mixtures containing approximately 5% CO_2 and different tensions of O_2. Data were corrected to a pH_e of 7.1. As a control coresponding data were determined for normal individuals. The position of the O_2 dissociation curve for healthy young adult males as determined by us was found to differ somewhat from reports in the literature. Furthermore the curve for normal children lay to the right of that for adult males. Positions of the curve for patients with cyanotic congenital heart disease, compared with those of normal individuals of approximately the same age, indicate a trend toward the right, i.e., toward decreased affinity of hemoglobin for oxygen. This is more marked in adults than in children, and is especially marked in cases complicated by pulmonary stenosis. This is interpreted as an adaptation to increase the oxygen tension of the tissues.

Hemostatic effects of normal blood transfusions in hemophilia-like swine. M. E. MUHRER (introduced by A. G. HOGAN). *Depts. of Agricultural Chemistry and Animal Husbandry, University of Missouri, Columbia, Mo.* Evidence has been accumulated to show that the hemophilia-like swine at the Missouri Experiment Station have a blood vessel defect in addition to the previously observed coagulation defect. A number of treatments have been effective in reducing the coagulation time to near the normal level but these treatments have not been effective in reducing the prolonged saline bleeding time. The injection of hemolytic agents or hemolyzed cells will sharply lower the coagulation time but has no effect upon the saline bleeding time. An intravenous injection of 5–50 ml. of normal blood in a 50-pound bleeder pig lowers the coagulation time but the bleeding time of over 600 seconds is not affected. However, if the amount of normal swine blood transfused into a 50-pound bleeder animal is increased to 500 ml. both the coagulation time and saline bleeding time are lowered. Ten animals ranging from 13 pounds–140 pounds in weight were given transfusions ranging from 175 ml.–1000 ml. All animals had a saline bleeding time of more than 600 seconds before transfusion and an average of 195 seconds after transfusion. It appears that there are two blood factors involved in the hemostatic defect in hemophilia-like swine, one that affects coagulation time and another that affects bleeding time. If there is a capillary defect, as is usually postulated with prolonged bleeding times, then the capillaries may be affected by the blood that is flowing through them and failure of the capillaries to constrict could be caused by a blood defect.

Relation of the electrolyte compositions of serum and skeletal muscle. EDWARD MUNTWYLER, GRACE E. GRIFFIN (by invitation), GEORGE S. SAMUELSEN (by invitation) and LOIS E. GRIFFITH (by invitation). *Dept. of Biochemistry, Long Island College of Medicine, Brooklyn, N. Y.* The electrolyte content of the serum and skeletal muscle was determined in rats and dogs maintained on diets deficient in potassium, and following repeated injections of desoxycorticosterone acetate. In rats, the loss of skeletal muscle potassium and gain of intracellular sodium was found to be accompanied by an increased serum bicarbonate and a lowered serum chloride concentration. These observations are in agreement with those reported recently by Darrow, *et al.* (*J. Clin. Invest.* 27: 198, 1948). In the present experiments the change in the composition of the muscle with respect to potassium and sodium was generally found to be less pronounced in dogs than in rats. Dogs with a deficit of muscle potassium and gain of intracellular sodium failed to exhibit the large increase in the serum bicarbonate concentration observed in rats. Similarly, there was a smaller decrease in the serum chloride concentration. However, in certain experiments with dogs the serum bicarbonate concentration tended to be increased. In these instances the serum chloride concentration appeared to show a relatively greater fall.

New colorimetric reaction for the determination of epinephrine. SAMUEL NATELSON and JOSEPH

B. PINCUS (introduced by ALBERT E. SOBEL). *Dept. of Chemistry and Pediatrics, Jewish Hospital, Brooklyn 16, N. Y.* 100 μg. of epinephrine heated in a boiling water bath for twenty minutes with 3 ml. of a 2% selenic acid solution in 1 N sulfuric acid will reduce the selenic acid to produce a pale blue color turning to yellow and then orange. An orange precipitate forms on standing. When this solution is cooled to room temperature and acid arsenomolybdate is added the molybdate blue develops in a few minutes. Acid Phosphotungstate and acid Phosphomolybdate will also produce blues but of lesser intensity. Glucose, Tryptophane, Glycine, Methionine and Tyrosine do not interfere in this procedure. Catechol gives the same reaction. The color read at 730 mμ. in the Beckman Spectrophotometer yielded an $E^{1\%}_{1\,cm.}$ of approximately 2000 at 730 mμ. based on the amount of epinephrine present. If the color is developed in 1 ml. and the micro cuvettes with a 1 cm. light path are used the procedure is capable of determining 0.2 μg. of epinephrine.

Concentration of bound pantothenic acid. J. B. NEILANDS (by invitation), HARVEY HIGGINS (by invitation), and F. M. STRONG. *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* The development of Lipmann's enzymatic method for liberation of pantothenic acid from coenzyme A preparations (*J. Biol. Chem.*, 167: 869, 1947) offered a convenient guide for attempts to purify the bound (*i.e.* microbiologically unavailable) form of pantothenic acid present in certain fresh biological meterials. Preliminary work with hog liver and fresh brewer's yeast involving hot water extraction, precipitation with 70% alcohol, and fractional precipitation with heavy metal salts led to preparations containing only about 0.1% pantothenic acid in the bound form. Better results were obtained when livers from freshly killed rabbits were homogenized with 0.9% KCl, and the easily sedimentable fraction ('cyclophorase', Green *et al.*, *J. Biol. Chem.*, 172: 389, 1948) was used as starting material. When this material was deproteinized with trichloracetic acid, and precipitated with barium and alcohol, the precipitate contained 0.2–0.4% of bound pantothenic acid. Counter-current fractionation (Craig) of such concentrates with phenol and aqueous buffer solutions raised the purity to about 2%. This represents approximately a 150–200-fold concentration as compared to the original liver. Such preparations contain no detectable free pantothenic acid.

Effect of *p*H and temperature on carboxypeptidase activity. HANS NEURATH and ELAINE ELKINS-KAUFMAN (by invitation). *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.* Kinetic measurements of the hydrolysis of carbobenzoxyglycyl-L-phenylalanine by carboxypeptidase were carried out at *p*H 6.5, 7.5, 8.3 and 9.0, and at temperatures ranging from 9° to 32°. Hydrolysis rates were evaluated according to the two-step reaction mechanism and were expressed in terms of K_m, the enzyme-substrate dissociation constant, and k', the specific rate constant for the activation process (Elkins-Kaufman and Neurath, *J. Biol. Chem.* 175: 893, 1948). K_m was minimal at *p*H 7.5 and increased about 5 fold between *p*H 7.5 and 6.5, and about 2 fold between *p*H 7.5 and 9.0. k' was constant between *p*H 7.5 and 8.3, with a sharp increase below and a moderate decrease above this range. The calculated maximum proteolytic coefficient was highest at *p*H 7.5. Measurements of the temperature effects at each *p*H were interpreted in terms of the classical thermodynamic constants, ΔH, ΔS and ΔF, for the equilibrium reaction (step 1) and in terms of the corresponding constants for the activation process (step 2), as previously described for chymotrypsin (Kaufman, Neurath and Schwert. *J. Biol. Chem.* 177, in press). ΔH decreased markedly with increasing *p*H. However, a compensatory decrease in ΔS, the entropy of the equilibrium reaction, rendered ΔF, the free energy change, nearly independent of *p*H (about 1,600 cal/mole). Similar relations were found for the corresponding thermodynamic constants of the activation process ΔF* about 15,000 cal/mole). The significance of these data is discussed.

Histochemical demonstration of three groups of alkaline (*p*H 9.2) phosphatases using various substrates and inhibitors. WILLIAM NEWMAN (by invitation), IRWIN FEIGIN (by invitation), ABNER WOLF (by invitation), and ELVIN A. KABAT. *Veteran's Hospital, Kingsbridge; Departments of Pathology and Neurology, Columbia University and the Neurological Institute, New York City.* The Gomori histochemical technic for localizing alkaline phosphatases was studied using glycerophosphate, glucose-l-phosphate, creatine phosphate, yeast nucleic and adenylic acids, thiaminepyrophosphate hexosediphosphate, muscle adenylic acid and adenosinetriphosphate as substrates and with various inhibitors. Three distinct groups of alkaline (*p*H 9.2) phosphatases were recognized. *Group I* enzymes were seen with all substrates in cytoplasm and nuclei of many organs, notably, intestine, renal tubules and adrenal cortex. Enzyme action is inhibited by heat, M/4 glycine or arginine and M/100 KCN, and corresponds largely with previous histochemical findings. Optimal *Group I* staining appeared with all substrates except thiaminepyrophosphate and yeast nucleic acid. *Group II* enzymes are demonstrated in cytoplasm of cells lacking *Group I* with muscle adenylic acid and adenosinetriphosphate, but not with yeast adenylic acid or other substrates, suggesting specificity for the purine-ribose-5-phosphate linkage. *Group*

II enzymes occur characteristically in cytoplasm and nuclei of smooth muscle and some connective tissues. *Group III* enzymes occur in nuclei. Muscle and yeast adenylic acids, creatine and glycerophosphates are more rapidly split than the other substrates; these differences in rates of hydrolysis permit recognition of *Group III*. *Group II* and *III* enzymes are less affected by heat, glycine, arginine and KCN than are *Group I* faciliatating differentiation. Dialysates of autolyzed rat intestine and guinea pig kidney and adrenal enhance staining even with optimal Mg^{++} and inhibitory glycine concentrations.

Effect of folic acid, liver extract and vitamin B_{12} on hemoglobin regeneration in chicks. C. A. NICHOL (by invitation), A. E. HARPER (by invitation), L. S. DIETRICH (by invitation) and C. A. ELVEHJEM. *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* No change in hemoglobin level accompanied the growth response of chicks to injection of antipernicious anemia liver extracts or vitamin B_{12} when a corn-soybean basal ration was fed. However, chicks fed a folic acid deficient purified ration for 21 days developed a slight anemia. If a single injection of phenylhydrazine hydrochloride (2 mg/100 gm. body wt.) was given at this time a severe anemia developed within two days. Experimental treatments were then begun and hemoglobin levels were determined at 2- to 4-day intervals. Injection of liver extract or vitamin B_{12} alone did not cause any significant hemoglobin response. Folic acid alone produced a definite increase and pteroyl triglutamate and pteroyl heptaglutamate were equally as effective at equimolar dosage. The combined administration of folic acid and liver extract or folic acid and vitamin B_{12} induced a more rapid regeneration of hemoglobin than treatment with folic acid alone. The duration of the depletion period influenced this response. After 14 days, treatment with folic acid and liver extract did not differ from treatment with folic acid alone. After 21 or more days depletion, treatment with folic acid and liver extract consistently caused more rapid regeneration of hemoglobin than treatment with folic acid alone. Chicks continued on a folic acid-deficient diet or on a diet containing vitamin B_{12} and low levels of folic acid developed a characteristic paralysis. The response of these chicks to treatment with folic acid and related compounds will be reported.

Protein and amino acid requirements of the German cockroach. J. L. NOLAND (by invitation) and C. A. BAUMANN. *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* Cockroaches were fed semi-synthetic diets containing various amounts and kinds of protein, and the rates of growth and maturation were determined. The quantitative requirement for casein was met at a level of 30% casein plus 30% bulk, or by 45% casein in the absence of bulk, indicating a relatively high requirement for dietary protein. When various proteins were fed at 15% of the diet, fibrin was superior to casein, followed by lactalbumin, egg albumin, zein, gelatin, oxidized casein and amino acid mixtures, in decreasing order of effectiveness. The 'incomplete' proteins zein, gelatin, and oxidized casein supported only slow growth, although some roaches fed these proteins reached maturity after a 30-fold increase in body weight. Analyses of cockroaches which had been reared to maturity on diets containing oxidized casein revealed that normal amounts of tryptophane and methionine had nevertheless been formed, indicating the synthesis of these two 'essential' amino acids by the insect or by its associated microorganisms. It was concluded from these experiments that the protein and amino acid requirements of the cockroach must be quantitatively and qualitatively different from the corresponding requirements of the rat and chick.

Pteridines on hemapoiesis and cell proliferation. EARL R. NORRIS and JOHN J. MAJNARICH (by invitation). *Dept. of Biochemistry, Univ. of Washington, Seattle, Wash.* Using the technique previously described (*Am. J. Physiol.* 152: 179, 153: 133, 1948), the effect of vitamin B_{14}, xanthopterin, folic acid and products of the action of enzymes, upon xanthopterin and folic acid, upon blood regeneration in rats made anemic on a purified diet containing 1% sulfathiazole, was measured. The results obtained with xanthopterin and folic acid checked the results previously obtained. Vitamin B_{14}, and the products of the action of enzymes upon xanthopterin and folic acid were much more effective in alleviating the anemia and leukopenia in the rats than were xantopterin or folic acid. The results obtained *in vivo* substantiated the results reported previously for cell culture *in vitro*, where vitamin B_{14}, and the products of the action of enzymes on xanthopterin and folic acid were shown to be more effective than xanthopterin or folic acid. The effect of certain minerals upon the anemia will be discussed.

Phosphatase activity in homogenates of liver and other organs of the rat. ALEX B. NOVIKOFF (introduced by HAROLD B. PIERCE). *Dept. of Pathology, Univ. of Vermont College of Medicine, Burlington, Vt.* Freshly prepared rat liver homogenates were assayed for phosphatase activity with a variety of substrates in a *pH* range of 4.0 to 9.1. The activity curves obtained fall clearly into 2 classes. *Class I* includes yeast adenylic acid (A-3-P) and 2 esters widely used in phosphatase determinations, sodium beta glycero-phosphate, and disodium phenyl phosphate (ϕ-P). None of these is present in animal cells. Activity is optimum near *pH* 5, low at *pH* 7.4, and very low at *pH* 9.1. *Class II*

includes esters of physiological significance: adenosine triphosphate (ATP), muscle adenylic acid (A-5-P), glucose-6-phosphate (G-6-P), fructose 1,6 diphosphate (HDP), and glucose-1-phosphate (G-1-P). There is little activity at *p*H 5.0. At *p*H 9.1, there is high activity with ATP, A-5-P, and HDP, and low activity with G-6-P and G-1-P. Most significant is the very high activity obtained with all 5 of these esters at *p*H 7.4, which is presumably close to physiological. When tested with 2 esters from *Class I* (ϕ-P and A-3-P) and 2 from *Class II* (A-5-P and ATP), homogenates of spleen and kidney give essentially the same type of activity curves as liver does, with only the *Class II* esters being actively split at *p*H 7.4. However, kidney is highly active with all 4 substrates at *p*H 9.1. The *p*H activity curves of intestinal mucosa homogenates are strikingly different from those of the other organs tested. All 4 esters yield similar curves, and irrespective of whether they belong to *Class I* or *II* they are actively split at *p*H 7.4.

Enzymatic synthesis of *l*-malic acid by CO_2 fixation. Severo Ochoa, J. B. Veiga Salles (by invitation), and Priscilla J. Ortiz (by invitation). *Dept. of Pharmacology, New York University College of Medicine, New York City.* As previously reported (Ochoa, Mehler and Kornberg *J. Biol. Chem.*, 174: 979, 1948) the equilibrium of the reaction *l*-malate + $TPN_{ox} \rightleftharpoons$ pyruvte + CO_2 + TPN_{red}, catalyzed by an enzyme isolated from pigeon liver, can be shifted toward CO_2 fixation by a dismutation with the glucose-6-phosphate dehydrogenase system. The overall reaction glucose-6-phosphate + pyruvate + CO_2 = 6-phosphogluconate + *l*-malate thus obtained, has been further studied with highly purified preparations of the pigeon liver enzyme. As with the tricarboxylic system (Ochoa, *J. Biol. Chem.*, 174: 133, 1948) the reaction can be followed manometrically through the liberation of CO_2 from bicarbonate by the phosphogluconic acid formed, since the disappearance of CO_2 by fixation is balanced by the formation of a carboxyl group of malic acid. *l*-Malate was determined with the enzyme system from *L. arabinosus* which specifically and quantitatively converts it to lactate and CO_2 (Korkes, and Ochoa, *J. Biol. Chem.*, 176: 463, 1948). The isolation and identification of *l*-malic acid will be described. The disappearance of glucose-6-phosphate and pyruvate was determined enzymatically with 'Zwischenferment' and lactic dehydrogenase respectively. The rate of malate synthesis is proportional to the CO_2 tension. By combination of the isocitric and malic enzyme systems one obtains the reversible reaction *d*-isocitrate + pyruvate + $CO_2 \rightleftharpoons$ α-ketoglutarate + CO_2 + *l*-malate. The equilibrium position of this reaction favors the formation of malate.

Extraction and estimation of desoxypentose nucleic acid (DNA) and pentose nucleic acid (PNA) from plant tissues. Maurice Ogur and Gloria Rosen (introduced by H. G. Albaum). *Botanical Laboratories, Univ. of Pennsylvania, Philadelphia, Penna.* Pentosans and polyuronides in plant tissues invalidate the estimation of PNA by pentose assay. The use of the ultraviolet absorption at 260 mu., for the estimation of the nucleic acids extracted from tissues, has been studied in this laboratory. Perchloric acid, which does not absorb significantly in the ultraviolet, is used to extract the nucleic acid constituents from the tissue residue. Our data indicate that prolonged contact in the cold with perchloric acid extracts PNA but not DNA from the plant tissue residue. Perchloric acid at elevated temperatures extracts both PNA and DNA. Root tip segments of rye and corn were homogenized in ethanol, centrifuged, and the tissue residue extracted with a mixture of alcohol and ether, and then with cold, dilute perchloric acid. Two alternate procedures were followed after these preliminary extractions: *1*) The tissue residue was treated with hot perchloric acid. DNA was determined in the extract by the diphenylamine reaction and total nucleic acid by the ultraviolet absorption at 260 mu. RNA was estimated by difference; *2*) The tissue residue was treated with perchloric acid in the cold and RNA determined in the cold extract. The residue was now treated with hot perchloric acid and the DNA determined. All fractions were examined by photocolorimetric estimation of *1*) total and inorganic phosphorus *2*) total nitrogen *3*) pentose *4*) desoxypentose *5*) ultra-violet absorption at 260 mu.

Effect of biotin and choline intakes on liver storage of ingested cholesterol. Ruth Okey, Eleanor Risser Vernon (by invitation), S. Lepkovsky and R. Pencharz (by invitation). *Dept. of Home Economics and the Division of Poultry Research, Univ. of California, Berkeley, Calif.* To test the effect on liver cholesterol storage of varying choline and biotin intakes 250 young rats were given biotin deficient diets with and without 1% cholesterol. Protein content of the diets (about 20%) total lipid, salts and vitamin supplements other than choline, biotin, and for one group, inositol; were kept constant and adequate for growth. One diet contained egg white and another whole egg. One third of each group received no biotin, one a borderline supplement and the last an ample amount. Subgroups of the egg white series were given choline in amounts furnishing respectively 0.10 mg., 0.25 mg., 0.50 mg. and 1.0 mg/gm. of diet. The whole egg diet furnished about 7 mg choline/gr. diet. Rats were killed after 7 to 9 weeks on diet. None of them showed kidney damage. In the egg white-fed animals given ample biotin or placed on diet after biotin storage (at 80–90 gm.) decreases in liver cholesterol with the increases in

choline intake noted above were slight and inconsistent. Egg white fed rats placed on diet at 45 gm and allowed to develop borderline biotin deficiency stored maximum liver cholesterol (about 1.6%) with 0.5 mg. choline and 0.5 mg. inositol/gm. diet. One group of rats given 0.1 mg. choline/gm. diet stored less than half this amount. Without added biotin liver cholesterols of rats fed whole egg were as low as 0.3%; with ample biotin supplements they reached 1.7% in the same time.

Acetate metabolism and ergosterol synthesis in Neurospora. R. C. Ottke (by invitation), E. L. Tatum and S. Simmonds. *Dept. of Microbiology, Yale Univ., New Haven, Conn., and Dept. of Biology, Stanford Univ., Calif.* Ergosterol has been identified as the principal sterol of *Neurospora*. The incorporation of deuteroacetate into mold mycelium, fat, and sterol has been followed using the wild-type strain and a mutant strain (Y-2492), which requires acetate for growth. Since considerably more deuterium is incorporated in the mutant than in wild-type grown under similar conditions, the results support the conclusions obtained from growth experiments (Tatum and Garnjobst, unpublished) and indicate that the mutant, in contrast to wild-type, is unable to form acetate from sugar. The results also show that acetate is incorporated in increasing proportions in *Neurospora* mycelium (about 3%), fat (about 10%, and in ergosterol (about 20%). Thus acetate metabolism in the mold appears to resemble closely that in the rat as shown by Bloch and Rittenberg. In an attempt to determine the location of the incorporated deuterium in the sterol molecule, infra-red absorption spectra have been obtained for the deuterated ergosterol. The spectra of ergosterol isolated from *Neurospora* grown on deuteroacetate and on normal acetate in heavy water show distinct and different absorption peaks, the former sample at 2225 cm^{-1} and the latter at 2190 cm^{-1}. Neither peak is shown by normal ergosterol. Although there is some uncertainty in the interpretation of the infra-red spectra, the location of these absorption peaks suggests that deuterium given as acetate may appear on the more unsaturated carbon atoms in ergosterol and that given as heavy water may appear at more saturated centers; i.e. methyl and methylene carbons.

Electrophoretic studies of the proteins of plasma and ascitic fluid in cirrhosis. W. D. Paul (by invitation), J. D. Gordon (by invitation), Robert L. Dryer (by invitation) and Joseph I. Routh. *Depts. of Medicine and Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City, Iowa.* Marked deviation from the normal plasma protein distribution is an outstanding feature of cirrhosis of the liver. The lowered total protein and inverted A/G ratio is often accompanied by formation of protein-rich ascites. We have determined by electrophoresis the protein components of simultaneously collected samples of plasma and ascitic fluid taken from several patients over intervals of time ranging from 7 weeks to 6 months. The results show a close qualitative similarity between any plasma sample and the corresponding ascites sample, which is perhaps produced by the dynamic equilibria between the blood and the transudate. Certain quantitative differences are also evident. In every case the ascitic fluid is richer than the plasma in albumin, α_1-globulin and γ-globulin, while the plasma was richer than the fluid in α_2-globulin, β-globulin, and fibrinogen. The best available estimates of the molecular weights of the various protein fractions show they increase in the order albumin, γ-globulin, α_1-globulin, α_2-globulin, fibrinogen, and β-globulin. These values indicate that possibly the selective protein enrichment of ascitic fluid may be directly related to the molecular weight of the protein components, and that in cirrhosis altered vascular permeability may be as important as altered protein synthesis.

Metabolism of alpha-estradiol in vitro. W. H. Pearlman and R. H. DeMeio (by invitation). *Dept. of Biochemistry, Jefferson Medical College, Philadelphia, Penna.* Alpha-estradiol-17-hemisuccinate as dissolved in a Krebs-phosphate medium buffered at *p*H 7.4 and incubated with rat liver slices at 37° for 4 hours. The ratio of hormone to liver tissue was approximately 1 to 500 parts by weight; in all, 845 mg. of alpha-estradiol (as succinate) were used. On extraction and fractionation of the incubation mixture, 54 mg. of crude crystalline estrone and 329 mg. of crude crystalline alpha-estradiol were obtained. These crystalline products were purified; estrone was further identified by C,H analysis of the acetyl derivative. An unsuccessful search was made for beta-estradiol. Although an appreciable amount of strongly acidic phenols were obtained, this material yielded no crystalline fractions on chromatography. Most of the estradiol succinate had undergone hydrolysis during incubation and very little if any of the hormone had been conjugated (e.g. as sulfate or glucuronide). Evidently alpha-estradiol can be converted to estrone *in vitro*, a process which is also known to occur *in vivo*. Estrone is very likely not the sole metabolite *in vitro* inasmuch as only about half of the estrogenic substance incubated could be recovered. This view is supported by earlier experiments (DeMeio *et al.*) wherein the biological inactivation of hormone was practically complete—the ratio of estradiol to liver tissue was 1 to 5,000 parts by weight.

Electrophoretic and chemical analyses of plasma and its fractions in health and disease. H. Rowland Pearsall (by invitation) and Alfred Chanutin. *Biochemical Laboratory, Univ. of Virginia, Charlottesville, Va.* Plasma and 5 plasma

fractions obtained by *Method 6* of Cohn *et al* were analyzed for their electrophoretic distribution and protein nitrogen, total lipide carbon and choles terol contents. Data are presented for normal individuals and for patients with diseases involving the kidneys, liver, and lungs. General trends are observed without the development of any characteristic pattern for these conditions. The plasma and fractions of multiple myeloma cases present specific electrophoretic patterns. The variations in the electrophoretic and chemical analyses are non-specific and do not yield sufficient information for diagnosis.

Comparative studies in niacin metabolism. W. A. PERLZWEIG, FRED ROSEN (by invitation), I. G. LEDER (by invitation), S. HUNTER (by invitation) and P. B. PEARSON. *Dept. of Biochemistry, Duke Univ., Durham, N. C., and A. and M. College of Texas, College Station, Texas.* Data on the urinary excretion of the major known metabolites of niacin in various species after saturation with nicotinamide will be presented. In man approximately 90% of 7 mg/kg/day of nicotinamide was recovered in equal amounts of N^1-methyl-nicotinamide (NMN) and the 6-pyridone of NMN. The major end-product in the dog is NMN which accounts for 90% of 10–20 mg/kg/day. In the rat 50% of 100–200 mg/kg/day was recovered as NMN, 15% as free niacin, and only 3% as the 6-pyridone, leaving 30% unaccounted for. The excretion of NMN in the rabbit (100 mg/kg/day), guinea pig (200 mg/kg/day), goat (80 mg/kg/day), calf (50 mg/kg/day), sheep (50 mg/kg/day), and horse (25 mg/kg/day) is less than 1% of the dose. The rabbit and guinea pig excreted 3% as the 6-pyridone, whereas the goat, calf and sheep excreted less than 1% of this metabolite. After the ingestion of NMN approximately equal amounts of NMN and the 6-pyridone were found in the urine of man and the rabbit. However, in both species the sum of these metabolites accounts for only 50% of the dose. After ingestion of NMN, the rat excretes 50% unchanged and 3% 6-pyridone, leaving 47% unaccounted for. The recovery in the urine of a single oral dose of the 6-pyridone is approximately 70% (of 50 mg.) in man, 85% (of 10 mg.) in the rat, 90% (of 25 mg.) in the rabbit and 40% (of 25 mg.) in the guinea pig. These data establish the 6-pyridone as a major metabolite of excretion for man but not for the other species studied. The rabbit, guinea pig and the polygastric herbivora can metabolize large amounts of niacin to unknown end products.

Incorporation of radioactive amino acids into the protein of acellular liver homogenate fractions. ELBERT A. PETERSON (by invitation), THEODORE WINNICK, and DAVID M. GREENBERG. *Division of Biochemistry, Univ. of California Medical School, Berkeley, Calif.* A homogenate is employed which consists of equal parts of: *1*) cytoplasmic granules obtained by fractional centrifugation; and *2*) supernatant from a concentrated (1:1) homogenate centrifuged at 2600 × g. Component (*2*) is completely inactive in the absence of (*1*). Component (*1*) serves as the locus for the amino acid incorporation process. However, radioactive protein appears in higher concentration in (*2*) during incubations with C^{14}-labeled amino acids. Accordingly, the mixture of (*1*) and (*2*) gives best results. Component (*1*) is inactivated by freezing, (*2*) is not. 0.33 ml. of the mixture, containing 0.020 gm. protein, is incubated with either 0.4 μM of labeled glycine or 0.8 μM labeled DL-alanine at 37° in horizontally-rotated centrifuge tubes, flushed with oxygen. The incorporation process is stopped by the addition of trichloroacetic acid. The precipitated protein is centrifuged, washed repeatedly with trichloroacetic acid, and freed of lipid by extraction with hot alcohol-ether. The lipid contains bound radioactive serine, when labeled glycine is used in the incubations. After 1 hour, approximately 0.75 μM of glycine or 0.35 μM of alanine are incorporated per gm. protein. These values represent uptakes of 3.8% of the glycine and 1.8% of the L-alanine employed in the experiments.

Some factors which influence the synthesis of peptide bonds as catalyzed by papain. CORNELIUS W. PETTINGA (by invitation) and SIDNEY W. FOX. *Chemical Laboratory, Iowa State College, Ames, Iowa.* The effects of various experimental conditions upon peptide bond synthesis as catalyzed by papain have been studied. The use of citrate buffer concentrations higher than had previously been reported, was especially effective in increasing the amount of benzoylamino acid anilide formed from the acid and aniline. The *p*H-yield curves for the reactions were similar in form for both 0.1M and 1.0M buffer concentrations with the optimum *p*H being near 5.0; *p*H-activity curves of similar shape have previously been obtained by others for other substrates. With the amounts of substrates, enzyme, and activator held constant, the yield of anilide decreased as the volume of buffer used was increased, for those acylamino acids studied. When benzoylamino acids of different solubility behavior were used, viz., benzoyl-DL-phenylalanine and benzoyl-DL-alanine, the effects of varied experimental conditions were nevertheless qualitatively similar. Six days' incubation gave only slightly greater yields than did 3 days' incubation; commerical papain catalyzed almost as well as a hydrogen sulfide-treated preparation; greater differences resulted when a more concentrated buffer was used, and from use of a higher ratio of aniline to benzoylamino acid. In the case of N-benzoyl-phenyl-alanine another important factor was the type of side-chain substitution. Related behavior

of substrates containing the same amino acid residues has been studied.

Oxalacetate decarboxylase of Azotobacter vinlandii. G. W. E. PLAUT (by invitation) and HENRY A. LARDY. *Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wis.* The enzyme oxalacetate decarboxylase was isolated from acetone dried cells of *Azotobacter vinlandii* by precipitation of inert proteins at *p*H 4.8, adsorption on copper hydroxide gel, elution with 0.1M *p*H 6.8 phosphate buffer and fractionation with ammonium sulfate. An increase in activity of 40–80 fold over that of the original aqueous extract of bacterial acetone powder was achieved. The purified decarboxylase was activated by Mn^{++}, Co^{++}, Zn^{++} and slightly by Mg^{++}. Pyrophosphate, A.T.P., and orthophosphate acted as inhibitors in the presence of Mn^{++}. When Co^{++} was the activator orthophosphate was not inhibitory. The inhibition by orthophosphate in the presence of Mn^{++} could be competitively reversed by Co^{++} indicating the existence of an inactive phosphate-manganese-enzyme complex. Co^{++} seems to compete with the phosphate-manganese radicle for the same point of attachment on the enzyme. Malate inhibited when either Mn^{++} or Co^{++} were used as activators. No fixation of $C^{14}O_2$ into oxalacetate could be demonstrated in the presence of the purified enzyme. Neither was the incorporation of radioactive carbon dioxide stimulated by A.T.P., fumarate, orthophosphate, or pyruvate either singly or in combination. The lack of fixation indicates that this enzyme merely catalyzes the equilibriumreaction: oxalacetic acid $\leftrightharpoons$ pyruvic acid + CO_2 ($K = 4.9 \times 10^3$) and is free of coupling enzyme systems. The biotin activities for *L. arabinosus* of enzyme fractions in various stages of purity were found to decrease with increasing purity.

Biosynthesis of carotenes in tomatoes. JOHN W. PORTER and RALPH E. LINCOLN (introduced by F. W. QUACKENBUSH). *Depts. of Agricultural Chemistry and Botany and Plant Pathology, Purdue Univ., Lafayette, Ind.* Several carotenes and 3 colorless polyenes have been found in small quantities in fruit of *Lycopersicon* species and strains. By breeding, it has been possible to obtain selections containing an increased content of 1 or more of these compounds. Chemical analysis of the more prominent colorless polyenes and carotenes has shown the structural similarity between these compounds and the well known carotenes. It is suggested that these compounds are intermediate steps in the synthesis of lycopene and *beta*-carotene. None of these compounds are found in quantities exceeding the quantity of lycopene in commercial tomatoes. Genetic studies have shown that a single gene difference exists between tomatoes containing lycopene and those yellow tomatoes containing little or no lycopene and only 2–3 μg. of carotene per gm. of fruit. A single gene difference also exists between tomatoes containing lycopene and those containing predominantly *zeta*-carotene and prolycopene. Another single gene difference exists between tomatoes containing lycopene and those containing *beta*-carotene. From the chemical and genetic studies, it is postulated that the synthesis of carotenes in tomatoes proceeds from simple compounds through *zeta*-carotene to lycopene and then to *beta*-carotene. The chemical steps for the postulated scheme involve dehydrogenation to form lycopene, followed by ring formation to yield *beta*-carotene. The scheme probably does not apply to carotene formation in green leaves.

Oxidative phosphorylation without deamination in mitochondrial preparations. VAN R. POTTER and W. C. SCHNEIDER (by invitation). *McArdle Memorial Laboratory, Medical School, Univ. of Wisconsin, Madison, Wis.* In isotonic homogenates phosphorylation could be coupled with the oxidation of mixtures of pyruvate and oxalacetate or fumarate via the Krebs cycle provided the reaction mixture was fortified with magnesium ions, inorganic phosphate, and adenosine triphosphate (ATP) or muscle adenylic acid (AMP). During the period of active inorganic phosphate uptake, the rate of NH_3 liberation was very slow, but when the inorganic phosphate concentration began to rise following a decrease in either substrate or enzyme activity, the output of NH_3 began to accelerate and continued until the NH_3 formed was equal to the amount of ATP added. In these experiments the amount of whole homogenate per flask was low, usually 50 mg. wet weight of tissue per flask. When homogenates in isotonic sucrose were fractionated by centrifugal means, the mitochondria from liver and kidney possessed considerably less than one-half of the oxidative activity of the whole homogenates, but if amounts of mitochondria were chosen so as to give an oxidative rate equivalent to what was obtained with the whole homogenate, the phosphate balance was maintained much longer with the mitochondria than with the whole homogenate and when phosphate output began, it was not followed by NH_3 output. The results conform with the view that ATP must be dephosphorylated before it can be deaminated. The presence of the deaminase appears to constitute an irreversible leak from the ATP-AMP system, and its separation from the mitochondria by centrifugal means facilitates the study of certain aspects of oxidative phosphorylation.

Water and electrolyte losses from skin of infants. EDWARD L. PRATT (by invitation), ROBERT E. COOKE (by invitation) and DANIEL C. DARROW. *Dept. of Pediatrics, Yale Univ. School of Medicine, New Haven, Conn.* By balance techniques the water loss from the lungs and skin at 80°F was about 48 gm/kg. of body weight per day. At 90° it was

106 gm. At 80° skin washings contained about 1 mM of chloride, 0.9 mM of sodium, and 1 mM of potassium per day. At 90° skin washings contained about 5 mM of chloride, 4 mM of sodium, and 6 mM of potassium per day. Calcium and phosphorus losses were negligible. The concentrations of electrolyte in sweat were calculated using 2 assumptions. First, all water losses from the skin at 90° were considered due to active sweating with no loss by diffusion. Therefore, the volume of sweat equaled the total water loss from the lungs and skin at 90° minus ⅓ of the insensible water loss at 80°. The loss of water via the lungs was considered to be constant in the 2 periods and approximately equal to ⅓ of the non-sweating insensible water loss. Second, skin washings at 80° were considered to arise from desquamation. The electrolyte in sweat equaled the skin washings at 90° minus the washings at 80°. The concentrations per kg. of sweat were about 5 mM of chloride, 4 mM of sodium, and 6 mM of potassium. These data indicate a low concentration of sodium and chloride in the sweat of normal babies. The concentration of potassium is high.

Metabolism of acetone. T. D. PRICE (by invitation) and D. RITTENBERG. *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.* Acetone labeled with carbon-14 in the methyl groups was administered to rats. From 0.2 to 1.1 mg. of the acetone was given by stomach tube. Little of the acetone is directly excreted, since only 0.6–10% of the acetone radioactivity was expired as carbonyl compounds. However, 50–80% of the activity appears in the respiratory carbon dioxide within 24 hours. The maximum activity in the expired carbon dioxide occurs about 2 hours after administration of the acetone; the activity of the carbon dioxide drops to a very low value after 12 hours. The administration of phenylaminobutyric acid resulted in the urinary excretion of a radioactive acetyl derivative. The activity of this product indicates that about half of the acetone was converted to acetate. In the normal rat, significant activity appeared in the carbon of the following compounds arranged in order of decreasing specific activity: liver cholesterol, bone carbonate, carcass cholesterol, carcass glutamic acid, liver glycogen, carcass aspartic acid, total carcass amino acids, carcass arginine and carcass fatty acids. No activity was found in the carcass leucine or tyrosine.

Partial separation of D- and L-peptidases and analogous dehydropeptidase I. VINCENT E. PRICE (by invitation), ALTON MEISTER (by invitation) and JESSE P. GREENSTEIN. *National Cancer Institute, National Institutes of Health, Bethesda, Md.* The enzymatic hydrolysis of glycyl-L-alanine, glycyl-D-alanine, and glycyldehydroalanine by aqueous homogenates of several normal rat tissues was studied. By means of high speed centrifugation (18,000 × *g* for two hours) a partial separation of the enzymatic activities was achieved. Between 75 and 95% of the original activity toward glycyl-D-alanine of homogenates of liver, kidney, spleen, brain, pancreas, testis and lung was associated with the sediment, whereas 80–95% of the original activity toward glycyl-L-alanine remained in the clear supernatant. About 80% of the original activity toward glycyldehydroalanine was also found in the supernatant in all of the tissues examined except kidney, in which 80–90% of the activity was associated with the sediment. Between 85 and 110% of the original activity was recovered in the pellet and supernatant fractions. The ratio of D-peptidase, L-peptidase, and dehydropeptidase activities in a preparation derived from kidney sediment was 100:53:122, while the ratio for the original kidney homogenate was 100:193:105. A preparation obtained from lung sediment possessed these activities in a ratio of 100:31:22, compared to 100:230:60 for the original homogenate. Thus in a variety of tissues D-peptidase activity may be readily separated from L-peptidase activity. These results suggest that hydrolysis of the 3 substrates studied may be catalyzed by different enzymes. The ratios obtained for the sediments support this hypothesis.

Fate of mercury in the rat following administration as mercuric acetate and phenylmercuric acetate. CLAUDIA S. PRICKETT (by invitation), EDWIN P. LAUG and FRIEDA KUNZE (by invitation). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* When phenylmercuric acetate and mercuric acetate are administered intravenously at a level of 120 μg. Hg per rat, marked differences in the excretion and storage of mercury occur. Thus, after mercuric acetate, 25% of the dose can be recovered in the kidney within 2 hours; only 4% after phenylmercuric acetate. In the liver, the situation is reversed, with phenylmercuric acetate favoring greater storage. In line with greater kidney storage after mercuric acetate is a significantly greater urinary excretion: in 48 hours this amounts to 17% of the dose for mercuric acetate, but only 3% for phenylmercuric acetate. The greater liver storage following phenylmercuric acetate is correlated with a prompt appearance of mercury in the small intestine, and a significantly elevated fecal excretion: in 48 hours this amounted to nearly 40% for phenylmercuric acetate, and only 10% for mercuric acetate. Oral administration, in contrast to intravenous results in much lower storage and excretion of mercury for both type of salts. However, phenylmercuric acetate is absorbed to a much greater extent than mercuric acetate, approximately 5 times as much mercury being stored in the liver and kidney, and twice as much excreted in the urine

Identification of L-ascorbic acid in the urine

of normal and chloretone-treated rats. G. W. PROBST (by invitation) and M. O. SCHULTZE. *Division of Agricultural Biochemistry, Univ. of Minnesota, Minneapolis, Minn.* Preliminary to other studies we have isolated and identified as L-ascorbic acid (M P 191°; mixed M P 191°; $[\alpha]_D$ 21° in methanol + 50.5; 174 mg. equivalent to 1 mM iodine; monoacetone derivative M P 220°; 228 mg. equivalent to 1 mM iodine) the indophenol reducing substance in urine of rats treated with 1,1,1-trichloro-2-methyl-2-propanol (Longenecker *et al. J. Biol. Chem.* 135: 497, 1940). Rat urine oxidized with 2,6-dichlorophenolindophenol or norite and treated with 2,4-dinitrophenylhydrazine (DNPH) under conditions prescribed by Roe and Kuether (*J. Biol. Chem.* 147: 399, 1943) yields mixtures of DNPH-derivatives from which that of L-dehydroascorbic acid can be isolated in pure form by chromatography on magnesium phosphate tetrahydrate. This derivative in 25 N H_2SO_4 has absorption maxima at λ528 mμ ($E^{1\%}_{1cm}$ 445) and λ370 mμ ($E^{1\%}_{1cm}$ 480) and a minimum at λ448 mμ ($E^{1\%}_{1cm}$ 212). The extinction ratios $E^{1\%}_{1cm}\frac{\lambda 530}{\lambda 450}$ and $E^{1\%}_{1cm}\frac{\lambda 370}{\lambda 450}$ serve as an excellent criterion of its purity. Depending upon the L-ascorbic acid content of the urine, the oxidant and the conditions of incubation, compounds other than L-dehydroascorbic acid yield DNPH derivatives which when dissolved in 25 N H_2SO_4 and at λ530 mμ account for 10–57% of the absorption of crude unchromatographed DNPH derivatives. The implications of this to methodology for determination of L-ascorbic acid will be discussed. From urine of normal rats on a milk diet we have isolated the pure DNPH derivative of L-dehydroascorbic acid. The latter, under suitable conditions, can be recovered quantitatively when L-ascorbic acid is added to urine.

Studies on lysozyme. CHARLES M. PROCTOR and SHERMAN R. DICKMAN (introduced by EMIL L. SMITH). *Dept. of Biological Chemistry, Univ. of Utah Medical School, Salt Lake City, Utah.* An assay for lysozyme consistent to 5% has been set up. The increase in transmission at 4400 Å of *Sarcina lutea* suspensions is measured after incubation with the enzyme, followed by addition of alkali. The effect of various agents on rate of lysis has been studied. No inactivation resulted from treatment of lysozyme overnight at 22°C. with 0.1M alloxan or 2.0M hydroxylamine, while 0.1M sodium hydroxide resulted in about 50% inactivation. Galston (1948, private communication) has observed an inactivation of several plant enzymes by incubation with riboflavin in strong light. A 40% inactivation of lysozyme has been obtained under similar conditions. Meyer and Hahnel (*J. Biol. Chem.* 163: 723, 1946) observed a striking increase in lysozyme activity by 0.2M sodium chloride when estimated viscosimetrically, but no effect on the rate of appearance of reducing sugars. This concentration of sodium chloride had no effect on the rate of lysis of *S. lutea*, whereas 0.5M sodium chloride gave about 50% inhibition and 1.0M sodium chloride 90% inhibition. Treatment of either lysozyme or *S. lutea* cells with high concentrations of sodium chloride had no effect on subsequent lysis.

Factors affecting the diazotization of bilirubin. BETTY KING PROFFITT (by invitation) and DEMPSIE B. MORRISON. *Dept. of Chemistry, Univ. of Tennessee, Memphis, Tenn.* The theory that serum bilirubin exists in more than one form is largely based on the difference in color developed when diazotization occurs in aqueous (direct reacting) and in alcoholic (total) solutions. The difference in color resulting from indirect reacting bilirubin. In an attempt to account for this difference we have investigated the diazotization of bilirubin over a wide range of conditions. Crystalline bilirubin dissolved in buffers, diluted human bile, a protein-bilirubin complex isolated from human bladder bile, and sera from patients with different types of jaundice and varying degrees of hyperbilirubinemia was used. The *p*H after diazotization of the above varied from 1–9. The concentration of nitrite in the diazo reagent was adjusted to optimum for any series and for a given *p*H. From the data to be presented it will be evident that the concentration of nitrite is highly critical for aqueous solutions. By adjustment of nitrite concentration it is possible to produce a biphasic reaction, maximum color and stability, fading colors or to suppress the development of color. Methanol solutions are less sensitive since the methanol appears to destroy the excess nitrite. Maximum color develops in aqueous solutions for all the materials tested at a *p*H 7–8 and optimum nitrite concentration. All sera so far examined when diazotized with optimum nitrite concentration give a direct/total color ratio of approximately 0.7.

Prothrombin consumption test. ARMAND J. QUICK AND JEAN FAVRE-GILLY (by invitation). *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.* On adding serum to a mixture of $CaCl_2$, thromboplastin and fibrinogen, a coagulation time is obtained which can be employed as a measure of the prothrombin remaining unconverted during coagulation in a test tube. The test has been standardized as follows. As a source of fibrinogen, normal plasma is used which is treated with $Ca_3(PO_4)_2$ to adsorb prothrombin (component B). To a mixture of 0.1 cc. of calcium phosphate-treated plasma, 0.1 cc. of 0.02 M $CaCl_2$, and 0.1 cc. of Quick's thromboplastin, 0.1 cc. of serum is added and the time required to form a clot accurately measured. The serum prothrombin

time (or prothrombin consumption time) for normal blood 1 hour after coagulation generally ranges from 16 to 35 seconds. In hemophilia it varies between 9 and 12 seconds and similar low values are found in severe thrombocytopenic purpura. The thrombin formed is inactivated so rapidly by the normal antithrombin that its effect on the test is not significant. Immediately after coagulation the serum prothrombin time is short (8–10 seconds) and remains constant for approximately 20 minutes or more before it begins to increase and finally reaches a maximum value. The increase in serum prothrombin time occurs sooner if the coagulated blood is centrifuged. The reason for this has not been determined.

Growth-promotion of yeasts by neopyrithiamin. JESSE C. RABINOWITZ (by invitation) and ESMOND E. SNELL. *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* Several yeasts are inhibited by thiamin when grown in the absence of vitamin B_6 (Schultz and Atkin. *Arch. Biochem.* 14: 369, 1947). *Saccharomyces carlsbergensis* 4228, commonly used for determination of vitamin B_6, shows a similar behavior. In the absence of vitamin B_6 or thiamin, 3.0 mg. of dry cells were obtained per cc. of culture medium. The addition of only 0.017 mγ of thiamin per cc. of culture medium caused 50% growth inhibition, and the addition of 1.7 mγ of thiamin per cc. completely prevented growth. This inhibition of growth by thiamin is alleviated by either vitamin B_6 or by neopyrithiamin, a growth-inhibiting analogue of of thiamin. Thus, in the absence of other data, neopyrithiamin might erroneously appear to act as an essential growth-factor for the yeast cells under these conditions. Inhibition of the organism by the addition of thiamin may result from increased synthesis of intermediate products, which may be toxic unless further metabolized. If vitamin B_6 were the limiting factor in this metabolic process, the addition of this vitamin would be expected to overcome the inhibition. Addition of neopyrithiamin would similarly overcome the inhibition since by acting as a competitive inhibitor of the added thiamin it could block formation of an excess of the toxic product. Thus, the antimetabolite would appear to be an essential growth factor, even though not involved in the normal metabolism of the cell. Reported instances in which organisms have come to require streptomycin or sulfonamides for growth may have a similar explanation.

Biosynthesis of heme. NORMAN S. RADIN (by invitation), D. RITTENBERG and DAVID SHEMIN. *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.* In an attempt to determine the precursors of heme we have incubated duck blood cells, both in plasma and buffer, with various compounds labeled with C^{14} and N^{15}. The mixture of preformed and newly synthesized heme was isolated as hemin and analyzed. Although the α-carbon and nitrogen atoms of glycine are utilized for porphyrin synthesis the carboxyl carbon atom is not. Comparison of C^{14} and N^{15} concentrations in hemin arising from glycine labeled in the α-carbon and nitrogen atoms suggests that more than 4 carbon atoms per hemin molecule are derived from the α-carbon atom. The carboxyl carbon atom of acetic acid participates in the synthesis of heme. Degradation of this hemin reveals that about half of the activity is in the carboxyl group. Some of the activity is in the rings containing the vinyl side chains (rings I and II). The hemin obtained from methyl labeled acetic acid is 6 times as radioactive as that obtained from carboxyl labeled acetate. The α-carbon atom of pyruvic acid is also utilized. The implications of these findings in relation to the biosynthesis of porphyrins will be discussed.

Effect of Theiler's GD VII virus on P32 uptake of one-day-old-mouse brain cultures. MAX E. RAFELSON, JR. (by invitation), HAROLD E. PEARSON (by invitation), and RICHARD J. WINZLER. *Depts. of Biochemistry and Bacteriology, Univ. of Southern California School of Medicine and Laboratory Division, Los Angeles County Hospital, Los Angeles, Calif.* The influence of Theiler's GD VII strain of mouse encephalomyelitis virus on the incorporation of P32-labelled phosphate into the phospholipid, total protein bound and organic acid-soluble fractions of minced one-day-old-mouse brain cultures incubated in Simm's solution at 35°C. has been studied. The total protein bound phosphate was fractionated by combining the methods of Schmidt and Thannhauser (*J. Biol. Chem.* 161: 83, 1945) and Schneider (*J. Biol. Chem.* 161: 293, 1945) yielding four fractions: ribonucleic acid, desoxyribonucleic acid, phosphoprotein and inorganic phosphate liberated from phosphoprotein. The virus-infected cultures, in comparison with non-infected cultures, had a markedly higher rate of P32 incorporation into the phospholipid, total protein bound and ribonucleic acid fractions in the first 6–24 hours of incubation. This is the period of maximal virus propagation. Phosphate turnovers in the organic acid-soluble, phosphoprotein, desoxyribonucleic acid and protein-liberated inorganic phosphate fractions were no different in the infected than in noninfected cultures. Both infected and noninfected cultures had P32 uptakes increasing up to 48 hours of incubation after which time the relative specific activities of the fractions decreased.

A new ganglionic blocking agent. LOWELL O. RANDALL, WILLIAM G. PETERSON (by invitation) and G. LEHMANN. *Pharmacology Dept., Hoffmann-La Roche, Inc., Nutley, N. J.* The d-3,4-(1′,3′-Dibenzyl-2′-keto-imidazolido)-1,2-trimethylenethiophanium d-camphor sulfonate (Nu-2222) is a

ganglionic blocking and vasodepressor agent which has about 30 times the potency and twice the duration of action of tetraethylammonium bromide. The vasodepressor effect is attributed to the blocking action on ganglia. Nu-2222 blocks transmission through the superior cervical ganglia since the reaction of the nictitating membrane to preganglionic stimulation is inhibited while the response to postganglionic stimulation is not affected. It blocks the vasodepressor response to vagus stimulation and the vasopressor response to carotid occlusion. The vasodepressor and ganglionic effects are counteracted by large doses of acetylcholine and Prostigmin. Ephedrine is an effective antidote for the vasodepressor action. Relatively large doses inhibit gastric and intestinal motility. There is little or no demonstrable curare-like action. Nu-2222 is 2 to 4 times as toxic as tetraethylammonium bromide by the intravenous route in mice, rats and rabbits but about 75 times as toxic in dogs. Lethal doses produce a hemorrhagic disorder in dogs which is accompanied by a prolonged clotting time. Such effects are not apparent in rabbits or cats. Nu-2222 has an approximate safety margin of 25 in dogs and 700 in rabbits.

Enzymatic mechanism of arginine synthesis. S. RATNER and ANNE PAPPAS (by invitation). *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.* It has been reported previously (S. Ratner, *J. Biol. Chem.* 170: 760, 1947) that rapid synthesis of arginine from citrulline occurs with partially purified enzyme preparations from extracts of beef liver acetone powder. The isolated system catalyzes the overall reaction: *L*-citrulline + *L*-aspartate + adenosinetriphosphate (ATP) $\rightarrow$ *L*-arginine + *l*-malate + ADP + H_3PO_4. The reaction has been found to proceed in several steps. Two distinct enzymes have now been obtained by fractionation with ammonium sulfate. One of these catalyzes a reaction between citrulline and aspartic acid to form an intermediate condensation product. ATP participates in the condensation reaction and inorganic phosphate is formed as citrulline is removed. A portion of the endergonic requirement of urea synthesis may therefore be associated with the formation of this condensation product, the necessary energy being derived from the high energy phosphate group of ATP. Magnesium ion is required and 0.01 M fluoride causes a 60% inhibition. The second enzyme catalyzes the hydrolytic cleavage of the intermediate accumulated in the first step, with the simultaneous appearance of arginine and malic acid. Experimental details relating to the enzymatic mechanisms and to the fractionation procedure will be discussed. Experiments on homogenates demonstrate that the above mechanism represents the main pathway of the conversion of citrulline to arginine in the biosynthesis of urea.

Studies on prolamines treated with nitrogen trichloride. I. Fractionation of hydrolysates. L. REINER, PHILIP WEISS (by invitation), FERNANDA MISANI (by invitation), M. G. CORDASCO (by invitation), and T. W. FAIR (by invitation). *Research Laboratories, Wallace & Tiernan Products, Inc., Belleville, N. J.* Finely divided zein and gliadin were reacted with NCl_3 vapors or by suspending them in a solution of NCl_3 in carbon tetrachloride. About 20 gm. of NCl_3 are used up per kg. of prolamine. The reacted prolamine produces typical running fits in rabbits in a minimum dose corresponding to 500 mg. N orally. Zein was hydrolyzed with pancreatin and with Protease 16 to an $^{-NH_2}/N$ value (formol titration) of 45–65% without impairment of its toxicity. It was then adsorbed to a cation exchange resin from which it was eluted with 2% pyridine. The eluate was toxic in a dose of 220 mg. N/kg. in rabbits, orally. Repeating this procedure with the first eluate yielded a second eluate which was toxic in a dose of 70 mg. N/kg. The second eluate was passed through charcoal previously conditioned with dilute acetic acid. The adsorbed material was eluted with large volumes of water. The combined eluate was toxic at a dose of 20 mg. N/kg. and some fractions of the eluate were toxic at a dose of 10 mg. N/kg. Part of the dried residue of the eluate was crystallized from EtOH. This was also toxic at 10 mg. N/kg. Its paper chromatogram (butanol-acetic acid) contained 5 spots suggesting that the product was not pure.

Studies on prolamines treated with nitrogen trichloride. II. Acetylated zein. L. REINER, FERNANDA MISANI (by invitation), PHILIP WEISS (by invitation), M. G. CORDASCO (by invitation), and T. W. FAIR (by invitation). *Research Laboratories, Wallace & Tiernan Products, Inc., Belleville, N. J.* Fractions which were isolated from zein treated with NCl_3 and which were toxic to rabbits in a dose corresponding to 36–220 mg. N/kg. were acetylated with acetic anhydride. The acetylated products did not produce running fits in dogs or rabbits when fed in doses 4 times as great as the minimum toxic dose of the unacetylated parent compound. Hydrolysis with 5N HCl reestablished their toxicity. Differences in the ability to acetylate *in vivo* may account for the great differences in the sensitivity of various species towards NCl_3 treated prolamines. Unhydrolyzed toxic zein was also detoxified by acetylation. Toxic zein lost its ability to produce fits when it was deaminated by diazotization. When zein was first acetylated, then treated with NCl_3, a product resulted which was as a rule less toxic, but it became more toxic upon deacetylation. These results suggest that a free $-NH_2$ group which is at the end of a peptide chain is essential for the elicitation of the toxicity but that blocking this group by acetylation does not interfere with the

formation of the toxic residue through reaction with NCl_3.

Determination of serum protein. Studies of agents for separation of albumin and globulin. JOHN G. REINHOLD, L. W. BLUEMLE, JR. (by invitation), FRANCISCO CAMPOY (by invitation), LOGAN GILMAN (by invitation), EDWARD F. GOULD (by invitation), VERNON L. MARTENS (by invitation) and RICHARD SHUMAN (by invitation). *Pepper Laboratory of Clinical Medicine and Nutritional Service, Hospital of the Univ. of Pennsylvania, Philadelphia, Penna.* Need exists for a rapid procedure for determination of serum albumin and globulin incorporating advances made recently in this field. A survey of agents proposed for separation of serum albumin and globulin was made by comparing the results with those of electrophoretic measurements at *p*H 8.6 and ionic strength 0.1. Sodium sulfate at a concentration of 26% and an approximate *p*H of 7.0 gave results approximating albumin and globulin values found by electrophoresis. Values obtained by use of sodium sulfite at 26.9% concentration were in fair agreement, while results obtained using 0.5 saturated ammonium sulfate (at a *p*H of 5.4 or 7.0), or methanol at 42.5% concentration *p*H of 6.8 and temperature of 1°C were less satisfactory. None of the salting-out methods were consistently in agreement with the electrophoretic separations. Attention to details of tecnique in the salting-out procedures appears important. The rapid separation method employing ether and centrifugation devised by Kingsley gave results similar to those obtained by filtration when 26% sodium sulfate was used. The applicability of the biuret reaction has been reevaluated. Determinations made by the biuret methods of Kingsley or Weichselbaum were compared with those of Kjeldahl or gravimetric methods. The means of all methods agreed closely, but occasional individual variations were marked. Discrepancies were least frequent between Kjeldahl and Weichselbaum biuret methods and the two may be used interchangeably for the usual serum analyses.

Influence of high levels of fat with suboptimal levels of riboflavin on the growth of chicks. RAYMOND REISER (by invitation) and P. B. PEARSON. *Agricultural and Mechanical College of Texas, College Station, Tex.* Chicks receiving moderately high levels of refined cottonseed oil (Wesson Oil) in diets low in biroflavin ceased to grow much sooner than those on a similar diet without added fat. The higher the cottonseed oil content of the diet the greater the inhibitory effect on the rate of gain. The addition of riboflavin to the diets at a level of 4 μg/gm. of feed prevented or corrected the phenomenon. Lard did not retard the growth rate on low riboflavin diets and a commercial hydrogenated vegetable oil to only a slight degree. The effect is not due to the development of rancidity in the high fat diets. The efficiency of utilization of the feed was lower on the cottonseed oil diets than on the low fat diets at low levels of riboflavin, but no differences were found in the riboflavin content of the livers.

Liver respiration as related to its xanthine oxidase activity. DAN A. RICHERT (by invitation) and W. W. WESTERFELD. *Dept. of Biochemistry, Syracuse Univ. College of Medicine, Syracuse, N. Y.* The determination of xanthine oxidase activity (Axelrod and Elvehjem) in a series of rat livers whose xanthine oxidase activity varied from 0–1800 units (Cmm O_2/gm/dry liver/hour) has demonstrated the following. *1*) The initial rate of the endogenous respiration of liver decreased 50% as the xanthine oxidase activity fell from 1600 units to zero. *2*) Tipping in xanthine to a respiring liver brei of low xanthine oxidase activity inhibited the endogenous respiration instead of increasing the oxygen consumption. *3*) Adding hypoxanthine to the brei at the start intensified the xanthine inhibition effect and prevented the detection of xanthine oxidase in livers with normal activity. *4*) Adding purified milk xanthine oxidase to livers of low activity restored the endogenous respiration to normal and prevented the inhibition otherwise caused by tipping in xanthine. *5*) Dialysis of the liver brei removed the substrates otherwise detected by adding purified xanthine oxidase, and also removed the xanthine inhibition effect. The results show that about 50% of the initial endogenous respiration in a normal rat liver homogenate is due to the oxidation of one or more substrates similar to hypoxanthine, and the relative accumulation of such substrates in a brei with low xanthine oxidase activity is responsible for the inhibition produced by added xanthine, in accordance with the known enzyme—substrate relationships for this system.

Utilization of human serum albumin injected intravenously. CECILIA RIEGEL and NICHOLAS GIMBEL (by invitation). *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* Human serum albumin was administered both orally and intravenously as the sole source of protein to 3 human volunteer subjects. Control periods using either milk or casein orally were also studied. Nitrogen, calcium, potassium, phosphorus and sulfur balances, changes in albumin-globulin ratio in the blood, blood and plasma volumes, thiocyanate space, nitrogen content of leg lymph and urea excretion were determined. Albumin administered orally was utilized in comparable fashion to milk and casein. On an oral intake of albumin of 12.0 gm. nitrogen daily 1 of 2 subjects was in nitrogen equilibrium; 1 had a daily negative balance of 1.6 gm. A 3rd subject was in negative balance on an oral intake of 8.0 gm. nitrogen daily. All 3 were in marked positive balance on an intravenous intake of either 8.0 or 12.0 gm. of albumin nitrogen daily.

From *1*) increase in total circulating plasma protein, *2*) excess circulating interstitial fluid albumin, as determined from thiocyanate space determinations and from analysis of lymph obtained from the leg, and *3*) amount of albumin excreted in the urine as albumin, we can, in *Patient S*, account for 774 of the 1100 gm. injected over a period of 22 days, leaving 326 gm. to be accounted for. If the difference between an assumed endogenous nitrogen excretion and the actually determined nitrogen excretion represents metabolic breakdown of albumin, the excretion of this amount of nitrogen in the urine is more than sufficient to account for the injected albumin not present in the body fluids. It is possible that this amount of albumin may have been metabolized during the 22 days of injection. That such breakdown is slow is indicated by the steady accumulation of albumin in plasma the longer the albumin injections were continued, by the low level of nitrogen excretion during the period when albumin was administered intravenously, and by a slowly increasing excretion of sulfur, which fell to a very low level when the subject was first changed from oral albumin to intravenous albumin.

Studies on the origin of serum proteins. SIDNEY ROBERTS (by invitation) and ABRAHAM WHITE. *Dept. of Physiological Chemistry, School of Medicine, Univ. of California at Los Angeles, Los Angeles, Calif.* Serum protein levels have been determined electrophoretically in the rat after either partial hepatectomy or abdominal evisceration. After partial hepatectomy, the earliest change was a decline in α_1-globulin, followed by later decreases in albumin and in the other globulins. Subsequently, the globulins, especially α_1-globulin, were rapidly restored. Albumin levels, however, remained low over the 48 hour experimental period. A maximum reduction in total serum protein concentration of 1.2 gm/100 ml. occurred 24 hours after partial hepatectomy. Following abdominal evisceration, the serum protein level dropped rapidly, falling 2.0 gm/100 ml. within the 23-hour experimental period. In this instance, the decline was due almost entirely to a diminution of albumin. Incubation of surviving liver mince in rat serum in an atmosphere of 95% oxygen, 5% carbon dioxide resulted in the liberation of large amounts of a protein with the electric mobility of serum α-globulin. The rate of liberation of this liver protein was initially as high as 30 mg/gm. of tissue per hour, but declined rapidly over the 3-hour incubation period. Similar experiments in a nitrogen atmosphere resulted in an increased rate of liberation of protein. In all experiments, a disappearance of albumin from the serum medium accompanied the release of 'α-globulin'. The results provide further evidence that the formation of blood proteins involves the integrated action of the liver and extrahepatic tissues.

Tissue collagen in experimental scurvy. WILLIAM V. B. ROBERTSON (introduced by H. B. PIERCE). *Division of Experimental Medicine and Dept. of Biochemistry, College of Medicine, Univ. of Vermont, Burlington, Vt.* The collagen concentrations in the skin and liver of normal guinea pigs, of those on a scorbutigenic diet and moribund with acute scurvy, and those on a scorbutigenic diet but maintained deficient for about 10 weeks with 0.35–0.15 mg. of ascorbic acid per day were determined. No changes in the collagen concentration in skin were found, whereas that in the liver was higher than normal in both scorbutic groups. The implications of these findings will be discussed.

Chemical changes in isotonic saline solutions placed in successively lower levels of human small intestine. C. S. ROBINSON, GLADYS R. BUCHER (by invitation) and D. DZIEWIATKOWSKI (by invitation). *Dept. of Biochemistry, Vanderbilt Univ., Nashville, Tenn.* The survey of the gradient in the small intestine altering saline solutions introduced at successive levels spaced 60 minutes apart, through a Miller-Abbott tube, has been continued using 50 cc. volumes per instillation of approximately isotonic solutions of Na_2SO_4, NaH_2PO_4 or Na_2HPO_4. Samples withdrawn 20 minutes after instillations were analyzed for total base, total CO_2, *p*H and the anions. The differences between successive pairs of samples (lower minus upper) were examined statistically. The average differences, with standard error, which represent the rate of change at intestinal levels 60 minutes apart as the tube advanced from pylorus to terminal ileum, are tabulated. A = number of samples, B = number of subjects, C = hours pylorus-terminal ileum, D = volume recovered in 60 minutes, E = mean difference in *p*H.

Saline used	A	B	C	D	E
NaCl, 50 cc. 150 mM	88	14	7–8	52.5 ±3 cc.	+0.186 ±0.03*
Na_2SO_4, 50 cc. 112 mM	28	8	4–5	63.7 ±3 cc.*	+0.290 ±0.05*
NaH_2PO_4, 50 cc. 104 mM	48	11	6–7	74.4 ±3 cc.*	+0.036 ±0.02
Na_2HPO_4, 50 cc. 101–143 mM	52	11	5–6	62.3 ±4 cc.*	+0.109 ±0.02*
Total Base	mM CO_2		Cl mEq/l.		SO_4 mEq/l. PO_4 mEq/l.
NaCl, +0.70 +0.28*	+3.80 ±0.63*		−3.32 ±0.80*		
Na_2SO_4, +1.02 ±2.11	+4.54 ±1.09*		−7.1 ±2.99*		+9.57 ±4.8*
NaH_2PO_4, +0.67 ±1.17	+0.82 ±0.24*		−9.9 ±2.80*		+9.3 ±6.9
Na_2HPO_4, +4.80 ±2.13*	+0.97 ±0.40*		−5.6 ±2.24*		+9.6 ±6.2

The differences marked * are statistically significant. Sulfate and phosphate ions suppress the entrance of chloride and this suppression is more pronounced as the ileocecal valve is approached.

Analysis of certain components of skeletal muscle during vitamin E deficiency. CHARLOTTE E. RODERUCK (introduced by H. A. MATTILL). *Dept. of Biochemistry, State Univ. of Iowa, Iowa City, Iowa.* In nutritional muscular dystrophy resulting from a deficiency of vitamin E, the free and total biotin content of skeletal muscle of hamsters, rabbits and guinea pigs did not vary from that of control animals. Reduction of the biotin intake (hamsters) decreased muscle biotin irrespective of the adequacy of the vitamin E intake. It is therefore unlikely that vitamin E acts as an antioxidant in tissue to preserve biotin, although this relationship has been observed in the autoxidative destruction of biotin by unsaturated fats in vitro. A sparing action of vitamin E on DPN was not demonstrated by mere addition of coenzyme I to Ringer phosphate medium in which dystrophic muscle strips were respiring; Q_{O_2} was not affected. Q_{O_2} values were also not influenced by addition of glucose, glutamic acid or phenylananine. Muscle strips from hamsters on vitamin E-deficient diets but not grossly dystrophic had Q_{O_2} values 33% higher than those from control animals. Differences of the same order were found in control and dystrophic guinea pig muscle. The glutamine content of skeletal muscles of dystrophic guinea pigs was about one-third that of muscle from control animals; the average content of non-glutamine amino acids was unchanged. In vitamin E-deficient rabbits, the difference was less marked. The possible significance of decreased glutamine levels is discussed briefly with reference to energy relationships. These results may be further evidence of an inefficient expenditure of energy possibly due to impairment in the process of phosphorylation.

Effect of a series of flavanoids on hyaluronidase activity. GERTRUDE RODNEY, L. M. WHEELER and ANN SWANSON (introduced by J. J. Pfiffner). *Research Laboratories, Parke, Davis & Co., Detroit, Mich.* The action of a series of flavanoids related to rutin on bovine hyaluronidase was studied *in vitro* by both the viscosity-reduction test and the turbidity-reduction method. Crystalline rutin was found to have no effect in concentrations of 5×10^{-4}M to 1×10^{-3}M on 10 units of enzyme but a product formed by pancreatic digestions showed some inhibition of the enzyme activity. Investigation of a series of 15 related flavanoids showed inhibitory activities which could be correlated with structural differences in the compounds. The action of these compounds *in vivo* on the spreading effect of hyaluronidase injected with hemoglobin in guinea pig skin was compared to the activity shown *in vitro*.

Studies in the metabolism of d-arabinose. JOSEPH H. ROE, EUGENE W. RICE (by invitation) and BENJAMIN W. SMITH (by invitation). *Dept. of Biochemistry, School of Medicine, George Washington Univ., Washington, D. C.* Following oral administration of D-Arabinose to rabbits, in doses of 2 gm/kg. of body weight, this sugar appears in the blood within ½ hour, reaches a peak level in 1-2 hours and disappears from the blood stream in 6-8 hours. The rate of absorption of D-Arabinose in the rabbit, determined by the Cori technique, was found to be 0.036 gm/100 gms. of body weight/hour. Balance studies after the oral administration of D-Arabinose, in which analyses of the alimentary tract, tissues, feces and urine were made, showed the following average total recoveries of this sugar: after 1 hour, 80%; after 2 hours, 67%; after 6 hours, 15%; after 12 hours, 9.5%; after 24 hours, 5.9 %. In the 24-hour analyses the pentose was found only in the urine. Analyses were made with the colorimetric method of Roe and Rice (*J. Biol. Chem.* 183: 507, 1948) which is based on furfural formation. These data show that D-Arabinose undergoes a metabolic transformation in the rabbit. *In vitro* studies of D-Arabinose added to homogenates of liver, kidney, muscle, blood and brain have shown a disappearance of this sugar after 13 to 24 hours' incubation at 37°C., under aerobic and anaerobic conditions. This disappearance is prevented by heating, iodoacetic acid and fluoride.

Hemolytic action of alloxan and alloxan derivatives. CATHARINE S. ROSE (by invitation) and PAUL GYÖRGY. *Nutritional Service of the Dept. of Pediatrics and the Gastro-Intestinal Section of the Medical Clinic, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* In the course of studies on the effect of dietary factors in alloxan diabetes it was found that alloxan caused marked hemolysis in rats receiving a tocopherol-deficient diet (*Science* 108: 716, 1948). The nature of this reaction has been investigated. Dialuric acid, alloxantin and ninhydrin caused hemolysis in the same manner as alloxan, ninydrin being the most active of the substances tested. The increased fragility of the red blood cells towards these compounds could be demonstrated after the rats had received the tocopherol-deficient diet for only 1 week. Alloxanic acid did not cause hemolysis. Studies were made *in vitro* using a suspension of red blood cells; all substances tested for hemolytic activity were prepared in phosphate buffer at pH 7.4. With dialuric acid the cells of tocopherol-deficient animals were hemolyzed. Alloxan was without effect, but treated with an equivalent amount of cysteine it behaved as dialuric acid. Larger amounts of cysteine inhibited hemolysis with either alloxan or dialuric acid. Various alloxan-related compounds and reducing agents have been studied. The red blood cells of animals receiving tocopherol were resistant to the action of dialuric acid and addition of tocopherol in about 1/50th the amount of dialuric acid protected the cells of tocopherol-deficient animals. These observations are in accord with an as-

sumption that tocoperhol interes with the formation of a free radical, a first oxidation product of dialuric acid, which is the actual hemolytic agent.

In vitro action of gentisic acid on hyaluronidase. SAUL ROSEMAN (by invitation), FAITH PEARSON (by invitation), and ALBERT DORFMAN. *Dept. of Pediatrics, Univ. of Chicago, Chicago, Ill.* The administration of sodium salicylate to rabbits inhibits the spreading effect of hyaluronidase (Guerra, F., *J. Pharmacol & Exper. Therap.* 87: 193, 1946). It has been demonstrated that salicylates do not inhibit hyaluronidase *in vitro* in concentrations that are achieved *in vivo* (Dorfman, Reimers, and Ott. *Proc. Soc. Exper. Biol. & Med.*, 64: 357, 1947; Pike. *Science*, 105: 391, 1947; Meyer *Physiol. Rev.* 27: 337, 1947). Meyer and Ragan (*Science* 108: 281, 1948) have recently claimed that this is due to the fact that salicylates are converted to gentisic acid which acts as the true inhibitor. Analytically pure gentisic acid was prepared by the oxidation of salicylic acid. Purification was achieved through lead salt fractionation and repeated recrystallization from water and ethyl acetate. Gentisic acid so prepared did not inhibit bovine testicular hyaluronidase. This confirms the findings of Lowenthal and Gagnon (*Canad. J. Research* 26: 200, 1948). Crude fractions were found to be inhibitory as was pure gentisic acid treated with alkali in the presence of air. Similar results were obtained with homogentisic acid. Thus the apparent inhibitory effect of crude gentisic acid is due to impurity which is probably an oxidation product. The possible significance of these results in the explanation of the *in vivo* effects of salicylates will be discussed.

Arginase, rhodanese ATPase activity of regenerating livers in protein-depleted rats. OTTO ROSENTHAL, CHARLES S. ROGERS (by invitation) and COLIN C. FERGUSON (by invitation). *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* Arginase, rhodanese and ATPase activities of regenerating liver following 70% hepatectomy have been studied in male 250-gm. Wistar rats maintained on a protein-free diet. Enzyme assays and determinations of the protein nitrogen were done upon aliquots of glass-homogenized tissue specimens. During 2 weeks of preoperative protein starvation the animals lost about 44% of the protein, 70% of the arginase, 65% of the rhodanese, and 50% of the ATPase of their livers. Thus while arginase and rhodanese activities of the liver protein were markedly diminished ATPase activity decreased but little. Following partial hepatectomy the concentrations of rhodanese and ATPase in the regenerating remnant continued to decrease slightly whereas the arginase concentration rose strikingly. The rise was apparent as early as 12 hours after hepatectomy. At 24 to 48 hours, i.e., at a time when only about 36% of the operatively removed protein was regenerated, the liver remnant contained as much or even more arginase than the entire preoperative liver. Smaller changes in arginase were found in livers examined 4 to 8 days after partial hepatectomy. The fluctuations of the liver arginase concentrations are probably a reflection of the changes in the endogenous protein metabolism which temporarily increases as a consequence of traumatic tissue breakdown and inadequate food consumption. No similar changes were observed in regenerating livers of rats kept on an 18% casein diet during the entire experimental period.

Hydrolysis of esters by rabbit polymorphonuclear leucocytes. R. J. ROSSITER and ESTHER WONG (by invitation). *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.* It has been found that washed suspensions of rabbit polymorphonuclear leucocytes, besides hydrolysing phosphate esters (*Biochem. J.* 43: xxi, 1948), also hydrolysed simple methyl esters of the aliphatic acids. That polymorphonuclear leucocytes hydrolyse tributryrin has been reported previously by Fleischmann (*Biochem. Ztschr.* 200: 25, 1928) and by Barnes (*Brit. J. Exper. Path.* 21: 264, 1940). These workers, however, referred to the enzyme as a lipase. It has now been shown that the enzyme is capable of hydrolysing simple triglycerides and simple methyl esters (up to C_8 have been tested), but not higher glycerides such as triolein. The enzyme should, therefore, be referred to as an esterase rather than a lipase. The rabbit polymorphonuclear leucocyte can hydrolyse neither acetylcholine, acetyl-β-methylcholine nor benzoylcholine and, therefore, contains neither 'true' cholinesterase, 'pseudo'-cholinesterase nor specific benzoylcholinesterase.

Studies on copper-containing oxidases in higher plants. M. L. ROTHCHILD and ROBERT MACVICAR (introduced by V. G. HELLER). *Depts. of Chemistry, Oklahoma A. and M. College and Agricultural Chemistry Research, Oklahoma Agricultural Experiment Station, Stillwater, Okla.* The distribution of polyphenol oxidase and ascorbic acid oxidase in leaf-blade tissue of several species of higher plants has been determined. Rapid oxidation of both catechol and 3,4-dihydroxy-DL-phenylalanine occurred in the presence of leaf tissue homogenates of several species of *Dicotyledonacae*. Of the plants studied tomato and sweet potato contained the most highly active enzyme system. Polyphenoloxidase activity could not be demonstrated in wheat leaf-blade homogenate. Ascorbic acid oxidase was present in sweet potato, soybean, and tomato leaf tissue; Swiss chard and wheat were less active. Sodium diethyldithiocarbamate in concentration of .001 and 0.01M did not appreciably inhibit O_2 consumption by leaf tissue homogenates in the presence of added DOPA; ascorbic acid oxidation was

markedly inhibited by both concentrations of this substance. The endogenous rate of O_2 consumption was not significantly affected by this inhibitor. Copper deficient tomato plants showed a marked decrease in polyphenoloxidase and ascorbic acid oxidase activity. The administration of copper ions resulted in rapid restoration of oxidative activity of these two enzyme systems.

Relationship of cell surface phosphatases to sugar metabolism in yeast. ASER ROTHSTEIN and REBECCA MEIER (introduced by H. C. HODGE). *Atomic Energy Project of the Univ. of Rochester, Rochester, N. Y.* It has been shown that the surface of the yeast cell contains phosphatases, which can dephosphorylate a large variety of compounds including phosphorylated sugars. The surface phosphatases can be completely inhibited by very low concentrations of molybdate or tungstate without any apparent injury to the cell. The inhibition is reversible and competitive in nature. It seems to be specific for the phosphatases. In the absence of molybdate, phosphorylated sugars can be metabolized at a slow rate which is limited by the rate of dephosphorylation. In the presence of molybdate, the phosphorylated sugars are not metabolized at all although glucose and fructose are consumed at a normal rate. It can be concluded, therefore, that phosphorylated sugars cannot be metabolized directly as such, but that the sugar residue can be metabolized after the phosphate group is split off. The function of the cell-surface phosphatases seems to be limited to the splitting of phosphate containing compound in the medium into utilizable residues, with no direct role in carbohydrate metabolism involved.

Inhibition of oxidation and phosphorylation in kidney homogenates by yeast adenylic acid. HENRY Z. SABLE (introduced by CARL F. CORI). *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo.* Various agents are known to inhibit aerobic phosphorylation in tissue homogenates. It was found that the oxygen consumption of washed kidney particles was increased to a maximum value, when, in addition to glutamate, Mg^{++} and fluoride, about 1 μM of muscle adenylic acid (adenosine-5′-phosphoric acid) was added per 2 ml. of reaction mixture. Increasing amounts of this nucleotide up to 12 μM caused a progressive increase in the uptake of inorganic phosphate from the medium, and correspondingly increased formation of acid labile phosphate compounds. When 6 μM of yeast adenylic acid (adenosine-3′-phosphoric acid) was added along with 12 μM of muscle adenylic acid, oxygen consumption was decreased, in a particular experiment, from 34 to 10 μM, uptake of inorganic phosphate was decreased from 775 to 107 μg. and formation of acid labile phosphate was decreased from 553 to 130 μg. In a similar experiment muscle inosinic acid (inosine-5′-phosphoric acid) in place of yeast adenylic acid had no inhibitory effect. The inhibitory effect of yeast adenylic acid on phosphorylation is probably due to competition with muscle adenylic acid for the enzyme which catalyzes the transfer of phosphate from intermediates formed during oxidation.

Formation of serine from glycine in the intact rat. WARWICK SAKAMI (introduced by HARLAND G. WOOD). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.* Glycine has been shown to condense with formate or a formate derivative to form serine. When C^{13} carboxyl-labeled glycine and C^{14}-labeled formate were administered to rats the liver serine was found to contain the C^{13} in the carboxy group and C^{14} largely in the β-position. In the present investigation we have studied the physiological formation of 'formate.' A possible mechanism is the deamination of glycine (Ratner, Nocito and Green) and oxidative decarboxylation of the resulting glyoxylic acid: glycine $\rightarrow$ glyoxylic acid $\rightarrow$ 'formate' + CO_2. To test the possible significance of this pathway in providing 'formate' for the conversion of glycine to serine we have administered glycine labeled with C^{14} in the methyl carbon to rats by stomach tube and determined the distribution of isotope in the liver serine and glycogen. The β-carbon of serine was almost (80%) as active as the α-carbon while the C^{14} was equally distributed between the 1,6 and 2,5 carbons of the glucose units of the glycogen. These results indicate that glycine itself may provide the formate or formate derivative involved in its conversion to serine. The data of Winnick, Moring-Claesson and Greenberg are in accord with this view. The β-carbon of their serine isolated from liver homogenate after equilibration with C^{14} methyl-labeled glycine contained a small amount of the isotope.

Tryptophane requirement of the rat as related to dietary level of other amino acids. W. D. SALMON. *Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.* The addition of 12% of acid hydrolysate of casein or an equivalent amount of gelatine to basal diets containing 7% of casein, .002% of niacin, and either 40% of corn grits or 7% of corn gluten meal depressed growth of rats. The growth depression was corrected by DL-tryptophane but not by additional niacin; .05% of DL-tryptophane added to the diet prevented growth depression or even supported growth at a higher rate than was obtained on the original basal diet. When the casein content of the basal diet was increased to 20%, the addition of gelatine depressed growth only if niacin was omitted from the diet. With niacin in the diet, this level of casein provided sufficient surplus tryptophane to balance the added gelatine. When 5% of glycine was added to the diets containing 7% of casein, growth was de-

pressed and the effect was not nullified by either niacin or tryptophane; the further addition of casein hydrolysate restored growth. Moreover, 5% of glycine added to a 21% casein basal diet, containing adequate niacin, did not depress growth significantly. It is apparent that, as the dietary level of other amino acids is increased, the essential requirement for tryptophane is also increased. The results with glycine suggest that the requirement for other essential amino acids may be similarly increased.

Effect of x-ray radiation and of starvation on hemoglobin synthesis in the rat. K. SALOMON, K. I. ALTMAN, G. W. CASARETT, and T. R. NOONAN (introduced by F. L. HAVEN). *Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* After whole body radiation and in acute starvation erythropoiesis is depressed. Information concerning the anabolism of the circulating hemoglobin under these conditions seems desirable. Since the alpha-carbon atom of glycine has been shown to be incorporated in hemin and globin, methylene labeled glycine was used in this investigation. Glycine (one or two microcuries) was administered to radiated and to starved rats. All animals were killed 24 hours later. Hemoglobin, hemin and glubin were isolated from their blood and the C^{14} content of these compounds determined. The C^{14} activity ratio of hemin to globin on a gram to gram basis was found to range from 4–13 in all control animals. The ratio of C^{14} activity of hemin to globin isolated from rats injected with glycine immediately after radiation with 600 r varied from 25 to 32. Hemin synthesis was increased in all these rats. On the other hand, when glycine was injected six days after exposure to 600 r no measurable hemin synthesis occurred, and the activity ratio was found to be zero. The activity ratio after five days starvation was 64; hemin synthesis in these rats was increased whereas globin formation was depressed. In seven days starvation the activity ratio was zero, hemin synthesis was not measurable, globin formation was depressed. In both radiated and starved animals the ratio of C^{14} activity of hemin to that of globin deviates considerably from the control values.

Effects of india ink injections in avitaminosis A. STELIOS C. SAMARAS (by invitation), NICHOLAS DIETZ, JR. (by invitation) and VICTOR E. LEVINE. *Dept. of Biological Chemistry and Nutrition, Creighton Univ. School of Medicine, Omaha, Nebr.* One hundred male rats were divided into several groups, some of which served as controls while others were allowed to develop the classical signs of vitamin A deficiency. Rats on the adequate stock diet and those on the vitamin A-deficient diet plus carotene tolerated injections of India ink. Rats on the vitamin A-deficient diet presented a sudden aggravation of the usual deficiency symptoms when injected subcutaneously with India ink. The rats injected in the afternoon, when examined the next morning, showed startling symptoms referable to the visual apparatus. They seemed nearly blind or totally blind, and could not find their food and water. Enophthalmos was marked, the decrease in the size of the eyeball being apparently due to loss of fluid. In vitamin A-deficiency blindness has a slow onset and is the end result of dryness, bacterial invasion and infection, bacterial damage or ulceration, and repair with formation of opacities. In vitamin A-deficiency aggravated by blocking or irritating the reticulo-endothelial system blindness occurs suddenly due to loss of physiological function, and is probably a depletion phenomenon. The phagocytosis induced by the introduction of the foreign body, India ink, may completely exhaust the vitamin reserve or may increase the metabolic demand for vitamin A to such an extent that hardly any of the small quantity yet available can be allocated to the retina for the rhodopsin-retinene cycle. Histological studies and analyses of tissues for carotene and vitamin A are in progress.

Relationship of purines, folic acid, and other principles in the nutrition of Leuconostoc citrovorum 8081. H. E. SAUBERLICH (introduced by W. D. SALMON). *Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn, Ala.* Further investigations on the nutrition of *Leuconostoc citrovorum* 8081 has revealed that the unknown factor appears to be involved in purine metabolism. The organism was found to require for growth either xanthine, guanine, or hypoxanthine. Adenine, thymine, and uracil had no apparent activity when tested alone, but did have a sparing action on xanthine, guanine, or hypoxanthine. Increased amounts of anti-pernicious anemia preparations, liver concentrates, rat urine preparations, or vitamin B_{12} concentrates could replace (in part at least) the requirements for guanine, xanthine, or hypoxanthine. High amounts of folic acid could, in the presence of purines, replace the unknown factor required by the organism. The omission of folic acid had no effect upon the response of the organism to the various concentrates of the growth principle. However, aminopterin at a level of 10 gamma/10 ml. assay tube inhibited growth completely. The inhibition could be reversed by anti-pernicious anemia preparations, liver concentrates, rat urine preparations, or thymidine. Folic acid was effective only when extremely high amounts were used. Rats injected with folic acid or fed diets containing 5 or 10 mg. of folic acid/kg. excreted in the urine a compound(s) active for the growth of *Leuconstoc citrovorum*. Sulfaguanidine fed to rats along with the folic acid did not decrease the activity of the urine. The feeding of vitamin B_{12} concentrates, ascorbic acid, or thymine in the place of folic acid in the

diets to rats did not cause an increase in the activity of the urine.

Effect of low environmental temperature on thyroid function as studied with radioactive iodine. H. G. SCHACHNER, Z. S. GIERLACH and A. T. KREBS (introduced by H. JENSEN). *Medical Dept., Field Research Laboratory, Fort Knox, Ky.* From studies with radioactive iodine, it was concluded by Leblond *et al* (Leblond, Gross, Peacock and Evans. *Am. J. Physiol.* 140: 671–676, 1943–44), that "exposure of rats to cold (0°–2°C.) for various periods of time produces a thyroid stimulation which is doubtful after 1–3 days, definite after 7 days, maximal at 26 days, but absent after exposure for forty days." A reinvestigation of the problem seemed indicated to show how early stimulation occurs and compares with the long time interval. Rats were exposed in individual cages to temperatures of 1°–4°C. and the uptake and organic binding of tracer and carrier doses of inorganic iodide after 1, 3, 7, 9, 26 and 60 days exposure were determined. A definite increased uptake of iodine was shown in the 1-, 3-, 7- and 9-day group. The results of these early time intervals will be compared with 26-, 40- and 60-day groups.

A study of tryptophane metabolism using isotope tracer technique. R. W. SCHAYER (by invitation), G. L. FOSTER, and DAVID SHEMIN. *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.* Tryptophane containing N^{15} in the indole nucleus has been synthesized and resolved into the D- and L- isomers. Tryptophane containing C^{14} in the β-position of the side chain has also been synthesized. After the intraperitoneal injection of N^{15} L-tryptophane into rats and rabbits, the isolated kynurenine, kyurenic acid and xanthurenic acid contained N^{15} having approximately the same concentration as the ring nitrogen of the tryptophane. Similarly, N^{15}-labeled kynurenine produced xanthurenic acid in rats without dilution. The 6-pyridone of N-methyl nicotinamide, isolated from rat urine with the aid of carrier after intraperitoneal injection of N^{15} DL-tryptophane, was found to have a small but significant N^{15} concentration. In contrast to this finding, the 6-pyridone isolated after incubation of N^{15} DL-tryptophane with rat liver slices, and after intraperitoneal injection of the C^{14} DL-tryptophane in rats, contained no excess isotope. Hemin isolated from rats after injection of large quantities of N^{15} DL-tryptophane had an insignificant N^{15} concentration. Studies are in progress on the fate of N^{15} indole, N^{15} L-tryptophane and N^{15} D-tryptophane in rats.

Determination of uric acid in whole blood and plasma. ARNOLD H. SCHEIN and EMILY F. RICE (introduced by HAROLD B. PIERCE). *Dept. of Biochemistry, College of Medicine, Univ. of Vermont, Burlington, Vt.* The determination of uric acid by the Folin method (or by its various modifications is beset with many difficulties which render un certain the values obtained thereby. Lack of speci ficity, inhibition of color development by variou substances and low recoveries of added uric aci have proved to be a great hindrance to accurate es timation of uric acid in biological material. Specific ity can be increased by employing uricase (Blauch Koch, Buchanan). Starting at this point we hav investigated the conditions necessary for the ac curate and precise determination of uric acid i blood and plasma. It was found that *p*H contro (*p*H 9.2—borate buffer) during color development i a requisite for good recoveries of uric acid. Folin Wu filtrates of many substances, notably protein and amino acids which cause color inhibition do s partially by virtue of their depressing effect upo the *p*H of the color reaction. True inhibitions o color development of the order of 10–20% caused by many amino acids in their expected normal (and abnormal) plasma concentrations, can be com pletely compensated by 'swamping' both the standards and the solutions containing inhibitors with adequate amounts of glycine. Experiments upon blood and plasma in which these new modi fications of the Folin method were employed revealed 90–95% recovery of added uric acid. Re coveries of the same order of magnitude were ob tained upon addition of known amounts of uric acid to uricase incubated blood and plasma (uric acid free). These results were checked by ultra violet enzyme spectrophotometry.

Intermediary metabolism of phenylalanine labeled with C^{14}. BERNARD SCHEPARTZ (by invitation) and SAMUEL GURIN. *Dept. of Physiological Chemistry, School of Medicine Univ. of Pennsylvania, Philadelphia, Penna.* Administration of DL-phenylalanine (labeled in the carboxyl and alpha positions) to phlorhizinized rats results in the appearance of C^{14} in the urinary ketone bodies as well as in the respiratory CO_2. Upon incubation of this type of phenylalanine with liver slices, acetoacetate labeled solely in the carboxyl group is obtained. When ring-labeled phenylalanine (carbons 1, 3, 5) is administered to phlorhizinized rats, C^{14} appears in the respiratory CO_2 as well as in the acetone moiety of the urinary acetoacetate. Incubation of ring-labeled phenylalanine with liver slices produces acetoacetate labeled chiefly in the terminal methyl group. The aromatic ring of phenylalanine readily undergoes cleavage in the course of metabolism, the immediate precursor of acetoacetate being a four-carbon unit derived from the ring and side-chain. Apparently no 2-carbon units are involved in this pathway. It is apparent that the alpha carbon of phenylalanine becomes the carboxyl carbon of acetoacetate while either carbon atom 1 or 3 of the ring forms the terminal carbon of acetoacetate. This furnishes direct proof tha

the side chain of phenylalanine shifts to an adjacent ring carbon during the formation of ketone bodies.

The quantitative determination of glycerylphosphorylcholine (GPC) in tissues. GERHARD SCHMIDT, L. HECHT (by invitation), N. STRICKLER (by invitation) and S. J. THANNHAUSER. *Tufts College Medical School, Boston, Mass.* Trichloroacetic acid filtrates of tissues are freed from interfering substances by precipitation with mercuric acetate at *p*H 5. (GPC and choline remain quantitatively in the supernatant.) Aliquots of the Hg free supernatant are precipitated with Reinecke salt before (A) and after (B) acid hydrolysis (N HCl; 20′; 100°). The reineckates obtained from the majority of tissues can be analyzed colorimetrically without further purification, but in certain tissues (intestinal mucosa) a reprecipitation of the concentrated acetone solution of the reineckate with aqueous Reinecke salt is necessary in order to obtain the choline reineckate in well crystallized form. The hydrolyzable choline (B-A) represents the choline group of GPC. The following amounts of GPC (mg. P/100 gm. fresh tissue) were found in fresh beef organs: liver 25; pancreas 15; submaxillaries 4; muscle, heart, brain, intestines negligible. Free choline: negligible in all fresh tissues. After 3 hours' incubation, a strong increase in the amounts of GPC occurs in pancreas (40), and strong increases of free choline, but not of GPC, occur in kidney and intestines. In incubated liver the amount of GPC remains practically unchanged, and no free choline appears in appreciable quantities.

Factors concerned in the liberation of pteroylglutamic (folic) acid from its heptaglutamate by chick liver. M. L. SCOTT (by invitation), C. H. HILL (by invitation), and L. C. NORRIS. *Cornell Univ., Ithaca, N. Y.* Previous results from this laboratory have shown that under certain conditions the activity of the vitamin B_c conjugase system in the livers from young chicks is increased by the addition of β-pyracin (4-pyridoxic acid). (*J. Biol. Chem.* 158: 291, 1945; *ibid.* 160: 265, 1945.) In a continuation of these studies, it has been found that livers from day-old chicks, produced by hens whose diet contained no animal protein, were nearly devoid of vitamin B_c conjugase activity. Under these conditions the effect of added β-pyracin was variable. On the other hand, the addition of certain concentrates derived from the alcohol-soluble material obtained in the preparation of a commercial liver extract produced marked increases in the liberation of folic acid from pteroylheptaglutamate. This effect was further enhanced when both the concentrates and β-pyracin were combined. In view of the suspected presence of small amounts of the antipernicious anemia factor in the liver concentrates, it was thought that vitamin B_{12} might be one of the factors required in the conjugase system. Accordingly, experiments were conducted in which concentrates of this vitamin were added to the liver incubation system with and without β-pyracin. Synergistic effects were also obtained between these two factors at a level of the vitamin B_{12} concentrate which provided approximately 4 $\mu\mu$M of vitamin B_{12}. Preliminary work using crystalline vitamin B_{12} have yielded similar results. The results obtained in these studies indicate, therefore, that this vitamin is required in addition to β-pyracin in the enzymatic release of folic acid.

Activation of purified prothrombin with sodium citrate. WALTER H. SEEGERS and ARNOLD G. WARE (by invitation). *Dept. of Physiology and Pharmacology, Wayne Univ., Detroit, Mich.* Purified prothrombin prepared by the method of Ware and Seegers (*J. Biol. Chem.*, 174: 565, 1948) is free of thromboplastin and Ac-globulin. The protein, when dissolved in 30% sodium citrate solution, is transformed to thrombin. At room temperature thrombin first begins to appear slowly after 16 hours, then rapidly for about 3 hours, and thereafter again more slowly. The form of the activation curve suggests autocatalysis. The prothrombin molecule apparently contains all of the necessary structural material for the formation of thrombin. The activation with sodium citrate can be completely blocked with small amounts of 3,4,4′-triaminodiphenyl sulfone or with 2-hydroxy-4,4′-diaminodiphenyl sulfone. All of the protein of the prothrombin preparation can be adsorbed on $BaCO_3$. The thrombin activity is not adsorbed on $BaCO_3$. Consequently purified prothrombin can be activated with sodium citrate, and any protein which is then not adsorbed on $BaCO_3$ must be thrombin alone or thrombin together with other parts of the original prothrombin. This provides an easy method to prepare thrombin. Moreover, all of the above facts are unusually valuable for gaining insight into the nature of prothrombin, its activation, and the nature of thrombin.

The estimation of glycogen in liver and muscle. SAM SEIFTER and SEYMOUR DAYTON (by invitation). *Dept. of Biochemistry, Long Island College of Medicine, Brooklyn, N. Y.* Dreywood (*Ind. Eng. Chem., Anal. Ed.*, 18: 499, 1946) described the use of anthrone for detection of carbohydrates, and Morris (*Science*, 107: 254, 1948) suggested its use for the estimation of glycogen. The present authors have employed the reagent in a simple direct method for glycogen in liver. In this method 4 to 8 gm. of liver (less may be used) are delivered into a weighed, graduated tube containing approximately 25 cc. of 30% KOH, and the tube reweighed. The tube contents are digested for 30 minutes in a boiling water bath, cooled, and made up to 35 cc. with KOH. Appropriate dilutions of the digest are

made with water (1:50, 1:250, etc.), and 5 cc. aliquots are transferred to Evelyn tubes. While submerged in cold water, the tubes then receive 10 cc. of 0.2% anthrone made up in 95% H_2SO_4. 5 cc. of standard containing 80 γ of glucose is similarly treated. All tubes are then heated for 10 minutes in boiling water, cooled, and read in the Evelyn photoelectric colorimeter at wavelength 620. The glycogen content of the liver is then calculated. When the glycogen content of liver or muscle is less than 0.6% this method is inapplicable due to interference of color produced by anthrone with the relatively increased protein contents of adequate aliquots. In these cases the glycogen may be first precipitated with alcohol, redissolved, and determined as above. The results agree with those obtained by the method of Good, Kramer and Somogyi.

Effects of ionizing radiation on the anaerobic CO_2 production and colony production in saccharomyces cerevisiae. FREDERICK G. SHERMAN and HERMAN B. CHASE (introduced by P. H. MITCHELL). *Brown Univ., Providence, R. I.* Yeast was grown (48 hours at 30°C.) in Reader's medium to which was added glucose 20 gm/liter and biotin, 2 micrograms/liter. Cells were washed twice with M/15 KH_2PO_4 or supplemented Reader's medium, diluted to standard density and dispensed into lusteroid centrifuge tubes. One half the tubes were irradiated 30 minutes (90,000r at 3000r/min). A 200 kv therapy tube operated at 20 ma was used as the source. After irradiation cell suspensions were transferred to small flasks and incubated at 30°C. for 2 to 24 hours. Aliquots were taken for plate counts and manometric estimation of anaerobic CO_2 production at stated intervals. Two to 4 hours after the beginning of irradiation the anaerobic CO_2 production (glucose or supplemented Reader's as substrate) is inhibited approximately 30% and about 85% of the irradiated cells are unable to form visible colonies. No inhibition of anaerobic CO_2 production was observed immediately after irradiation. Between 12 and 24 hours anaerobic CO_2 production on a per cell basis progressively declines suggesting that some 'injured' cells have further reduction in ability to ferment glucose, or have lost it entirely. Investigations by earlier workers on the effect of x-radiation on enzyme systems *in vivo* indicate that fairly large doses have practically no effect in diminishing their activity (Wels and Osann, *Pflügers Arch.* 207: 156, 1925). Only with yeast grown on Reader's medium plus biotin have we been able to obtain consistent and reproducible inhibition of fermentation after irradiation.

The biological synthesis of serine from glycine. PHILIP SIEKEVITZ. (by invitation), THEODORE WINNICK and DAVID M. GREENBERG. *Div. of Biochemistry, Univ. of California Medical School, Berkeley, Calif.* The *in vitro* synthesis of serine from glycine has been demonstrated both in rat-liver slices and homogenates by means of tracer experiments with C^{14}-labeled glycine. The reaction proceeds anaerobically. Employing carboxyl-labeled and methylene-labeled glycine, it has been shown that the carboxyl-carbon of glycine appears in the carboxyl-group of serine, while the methylene-carbon of glycine appears in both the alpha and beta carbons of serine. Aerobically, the C^{14} concentration of the beta-carbon was approximately 40% that of the alpha-carbon. Anaerobically, it was only 5%. Sakami (*J. Biol. Chem.* 176: 995, 1948) has shown that formate reacts with glycine to form serine, the formate carbon appearing in the beta-carbon of serine. We have now shown that this formate, at least in part, may be derived from glycine itself. Rat liver slices were incubated in nitrogen with radioactive glycine and non-radioactive formate, the latter at twice the concentration of the former. One flask (I) contained methylene-labeled glycine; the other (II) had carboxyl-labeled glycine. Carrier formate was added, steam-distilled, and oxidized to CO_2 (Osborn, Wood, and Werkman, *Anal. Ed., Ind. Eng. Chem.* 5: 47, 1933). Glyoxylic acid is not oxidized to CO_2 by this method. The CO_2 obtained from flask (I) was radioactive, that from flask (II) was not, showing that formate is derived from the alpha carbon of glycine. The dilution of radioactivity in the beta-carbon indicates a non-glycine source of formate, which is more active anaerobically than aerobically.

Oxygen consumption of 'Killer' and 'Sensitive' stocks of paramecium aurelia, variety 4. D. H. SIMONSEN (by invitation) and W. J. VAN WAGTENDONK. *Dept. of Zoology, Indiana Univ., Bloomington, Ind.* Two genetically diverse stocks occur within variety 4 of Paramecium aurelia. The 'killer' stocks produce a toxic substance, 'paramecin', which kills 'sensitive' animals in a typical manner. The two types are determined genetically. The killer stocks possess the killer gene K which can support the multiplication of the self-duplicating cytoplasmic factor, 'kappa', but cannot initiate its production *per se*. Kappa in turn controls the production of paramecin. Sensitive animals may be those having the genotype KK without kappa, or those with the recessive allele kk. Sensitive animals are killed in a typical fashion when mixed with killer animals, when placed in culture fluid in which killers have grown, or when exposed to a dilute brei prepared by crushing killer animals. Measurements of the rates of oxygen consumption of these stocks were made using the Cartesian diver micro-respiration technique. The stocks used for comparison belonged to the same mating type and were grown under the same conditions of feeding. The live animals were suspended in sterile 'exhausted' lettuce infusion in the divers. Rates of oxygen consumption were determined over a period of 8–10 hours at 27°

Killer animals were found to respire at a higher rate than sensitive animals. The respiration of sensitive animals while under the influence of paramecin was followed. Pertinent data will be presented.

Serum lipides of Eskimos. Effect of a high fat diet (pemmican) and of fasting. R. G. SINCLAIR, G. M. BROWN (by invitation) and L. B. CRONK (by invitation). *Depts. of Biochemistry and Medicine, Queen's Univ., Kingston, Canada.* As part of a clinical and biochemical study of the Eskimos on Southampton Island in the Eastern Canadian Arctic, lipides were determined in the serum of 126 persons. Regardless of age, sex and absorptive state, the average values, in mg/100 ml., were: Total fatty acids, 329 ± 103 (S.D.), lipide P, 9.9 ± 2.0, total cholesterol, 173 ± 29, free cholesterol, 47.5 ± 9.4. Free cholesterol amounted to 27.7 ± 3.0% of total. The molar ratio of phospholipide to total cholesterol was 0.72 ± 0.09. A palpable hepatomegaly was observed in about ⅓ of the Eskimos. The average values for the serum lipides in this group were the same as in those without enlarged livers. In a small group of 2 men and 2 women who subsisted for 6 days exclusively on pemmican (75% of the calories from beef fat), the levels of all lipides increased considerably. A subsequent 4 day fast by 3 subjects caused a further appreciable increase in all lipides —total fatty acids, 55% increase, lipide P, 30%, total cholesterol, 15%. A similar fast by a man who had been on his customary diet of meat and bannock caused an increase in total fatty acids but not in lipide P, total or free cholesterol. Little or no ketonuria developed after 6 days on pemmican; the subsequent fast caused a definite ketonuria.

The lipotropic action of threonine. S. A. SINGAL, V. P. SYDENSTRICKER (by invitation) and JULIA M. LITTLEJOHN (by invitation). *Univ. of Georgia School of Medicine, Augusta, Ga.* While studying the nicotinic acid-tryptophan relationship in rats on 9% casein ration containing 5% fat and 0.2% cystine and choline, yellowish livers were observed at autopsy. Upon analysis the liver lipids were 16.0%. Supplementary nicotinic acid, tryptophan, valine, histidine, phenylalanine or serine were without effect. The addition of threonine reduced the liver lipids to 5.1%. Because of the growth depressing action of threonine under these experimental conditions (*J. Biol. Chem.* 176: 1063, 1948), nicotinic acid or tryptophan was added to this diet under which conditions growth was satisfactory and the liver lipids were 6.6%. When choline was increased to 1.0%, a similar reduction of liver lipids was observed. That the lipotropic action of threonine was not the result of increased food intake was demonstrable in paired-feeding experiments. Here the liver lipids of rats on the casein ration supplemented with tryptophan was 14.4% as compared to 5.9% when threonine was also included. When the dietary fat was increased to 40%, the liver lipids were 21.8 and 10.1% respectively. The livers of rats on an amino acid diet simulating the 9% casein ration supplemented with tryptophan contained 17.0% lipids. Supplementary threonine reduced the lipids to 5.3%. When threonine was omitted from the entire ration, the rats lost weight and the liver lipids were only slightly above normal.

Unlikelihood of specific long range forces in immunologic and enzymatic reactions. S. J. SINGER (introduced by L. ZECHMEISTER). *Gates & Crellin Laboratories of Chemistry, California Inst. of Technology, Pasadena, Calif.* Rothen has performed experiments (*J. Biol. Chem.* 168: 75, 1947) in which antibody is adsorbed on antigen films which are apparently covered with inert barriers, and other experiments (*J. Am. Chem. Soc.* 70: 2732, 1948) in which enzymatic reactions occurred despite apparent separations of enzyme and substrate proteins. From these he concludes that specific long range forces are involved in immunologic and enzymatic reactions. By 3 approaches, we have obtained evidence that holes in the barrier films are responsible for the effects observed. *1*) Certain cellulose derivatives used as inert barriers reduce the adsorption of antibody on films of bovine serum albumin considerably. Ethyl cellulose films cast from ethylene dichloride solution are particularly effective, but those cast from acetone solution permit increased antibody adsorption. This demonstrates the importance of the physical state of the barrier films. *2*) With the electron microscope, we find that antibody is concentrated in clumps when adsorbed on formvar films above bovine serum albumin layers, although antibody is evenly distributed when adsorbed directly on the antigen, and the surfaces of the formvar films without antibody are uniform. This suggests that reaction occurs through holes in the formvar films. *3*) Intervening formvar films permit antibody directed against the p-azophenylarsonic acid group to be adsorbed on p-azophenylarsonic acid conjugated proteins. Since the work of Landsteiner, Pauling and collaborators, among others, makes it extremely likely that these conjugated protein systems react by a short range mechanism, these experiments indicate that the antigen and antibody molecules actually come into contact through holes in the polymer films.

Inhibitors of the L-amino acid oxidase of snake venoms. THOMAS P. SINGER and EDNA B. KEARNEY (by invitation). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.* The L-amino acid oxidase of snake venoms, apparently a yellow enzyme, is inhibited by flavin analogues. The analogue, isoriboflavin, which has proved inhibitory to every yellow enzyme tested in this laboratory, inhibits the L-amino acid oxidase of moccasin venom 50% at 0.0004M concentration. Riboflavin itself inhibits appreciably even at

0.00004M concentration. 0.002M atabrine decreases the activity by 50%. A group of polyvalent anions are even more inhibitory to this enzyme. Notable among these are inorganic phosphate and arsenate, which completely inactivate the enzyme at 0.0001M or lower concentrations, under appropriate conditions. An extensive study of this enzyme in various venoms revealed that the oxidases of pit vipers are highly sensitive to phosphate, those of true vipers less sensitive, and those of colubrine venoms insensitive. While the action of riboflavin analogues on the enzyme is instantaneous, the effect of phosphate and arsenate increases with time and also with temperature, *p*H, and the molarity of phosphate; it is inversely related to the protein concentration. Monovalent anions and organic bases protect the enzyme from the action of phosphate, while substrates and riboflavin analogues protect in catalytic amounts. The inhibition is not of the competitive type. Metaphosphate, pyrophosphate, organic phosphates, arsenite, molybdate, sulfate, and thiosulfate are less inhibitory, but ferricyanide is as effective as phosphate. The kinetics of the reaction have been studied in detail, and a combination with the enzyme protein is suggested as a probable mechanism.

Phosphomannose isomerase. MILTON W. SLEIN (introduced by GERTY T. CORI). *Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis, Mo.* Lohmann's phosphohexose isomerase catalyzes the reaction, glucose-6-phosphate $\rightleftharpoons$ fructose-6-phosphate. A purified isomerase which can be prepared from rabbit muscle or from yeast does not act on mannose-6-phosphate. The latter was prepared by allowing crystalline yeast hexokinase to act on mannose in the presence of ATP. It was found that rabbit muscle contains a separate enzyme which together with Lohmann's isomerase establishes an equilibrium between the three phosphorylated hexoses in the approximate ratio of glucose 56, fructose 29, and mannose 15%.

Isolation of the staphylococcus stimulatory factor(s) (SSF). NATHAN H. SLOANE (by invitation) and RALPH W. MCKEE. *Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Staphylococcus albus grows maximally on a synthetic medium but with a lag phase of 12–16 hours. Growth is stimulated with extracts of vegetable, animal and bacterial products. Antipernicious anemia liver extract is inactive. The active factor(s) in deproteinated plasma and urine are non-dialyzable, heat stable, moderately stable to acid and alkali, stable to proteolytic enzymes, soluble in methanol (*p*H 3), insoluble in common organic solvents, precipitable with silver (*p*H 6) and inactivated by ninhydrin. Combining dialysis and silver precipitation the activity in human urine is concentrated 200- to 400-fold (nitrogen) with almost 100% recovery. The material is quite unstable upon standing but activity can be restored by reaction with glutathione. The electrophoretic pattern (*p*H 8.6) shows five peaks, the mobilities of two being within the range of α_1 and α_2 globulins, the other peaks had mobilities greater than the normal plasma constituents. Using bovine plasma, deproteinizing with heat (about *p*H 5), dialyzing and precipitating with silver, the activity is concentrated 140- to 400-fold (0.06–0.021 mg. N/U) with 50–100% recovery. The electrophoretic pattern (*p*H 8.6) shows 4 peaks, the mobilities of 2 being within the range of α_1 and α_2 globulins. This concentrate gave a positive orcinol hexose test. Treatment of this concentrate with chloroform-isoamyl alcohol (*p*H 6.6) causes a 90–100% loss of activity, which is restored with glutathione or cysteine. The degree of purification is 850- to 1700-fold (nitrogen). The purified material is non-dialyzable. Human plasma and its protein fractions (Cohn *et al.*) were studied and fraction 1V-4 contained the greatest concentration of SSF.

Studies on transaminase in germinating seeds and its relation to protein synthesis. BETSY PATTERSON SMITH (by invitation) and HAROLD H. WILLIAMS. *Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.* The relative changes in the amounts of glutamic-alanine and glutamic-aspartic transaminase and protein were determined during the first 120 hours of germination of seeds of *Gramineae* (barley and corn), *Leguminosae* (pea and wax bean), and *Cucurbitaceae* (pumpkin and squash). All the seeds tested began germination with the same low level of transaminase, but the amount synthesized during growth ranged from 0.3 units in the pea to 1.33 units in barley for the glutamic-aspartic transaminase and from 0.20 units in squash to 0.70 units in barley for the glutamic-alanine enzyme. In almost every case the glutamic-aspartic transaminase increases more rapidly than the glutamic-alanine enzyme. In general, the rate of transaminase formation during germination was greater than that of protein as judged by the increased number of units of the enzyme per milligram of protein nitrogen. Supplying the germinating seeds with a nutrient solution caused an increased rate of synthesis of both transaminase and protein, but the change is greater for the enzyme than for protein. Thus it is indicated that transamination is limited in its role in the plant to the interconversion of amino acids and keto acids, and that the new amino acids formed may have several possible fates, only one of which is the synthesis into protein.

Optical and side-chain specificity of leucine aminopeptidase. EMIL L. SMITH and W. J. POLGLASE (by invitation). *Laby. for the Study of Hereditary and Metabolic Disorders and the Depts. of Biochemistry and Medicine, Univ. of Utah School of Medicine, Salt Lake City, Utah.* The side-chain

specificity of this enzyme is not absolute; a number of aliphatic amino acid amides are attacked at different rates. These rates were estimated from the first-order coefficients obtained with two different preparations of the enzyme, one having 20 times the activity of the other. Nevertheless, the relative rates for the 2 preparations for the following compounds were identical: L-leucinamide (100), DL-norleucinamide (100), DL-norvalinamide (80), DL-α-n-aminobutyrylamide (44), L-alaninamide (6.3), and glycinamide (0.34). Only the L forms are hydrolyzed; D-leucinamide, D-leucylglycine, and D-leucyl-L-tyrosine are completely resistant to the enzyme. L-Leucyl-L-alanine is split about 25 times faster than L-Leucyl-D-alanine. Both bonds of L-alanyl-L-leucinamide are rapidly hydrolyzed, and the rate is about 850 times greater than the action on D-alanyl-L-leucinamide where only the terminal amide linkage is split. β-Alanyl-L-leucinamide is hydrolyzed even more slowly than is the latter compound. An interpretation of the action of this enzyme has been made in terms of the polyaffinity theory. The substrate combines with the protein through the metal (Mn^{++} or Mg^{++}) at the free amino group and at the nitrogen of the sensitive amide or peptide linkage. The residue which bears the carbonyl of the sensitive bond has its side-chain bound directly to the protein; the affinity of the protein for the side-chain is a function of the mass of the aliphatic group. The enzymatic specificity thus depends on correct steric relationships of the side-chain and of the groups which are bound to the metal.

Interrelationship between chloramphenicol (chloromycetin) and enzymatic systems. GRANT N. SMITH and CECILIA WORREL (introduced by O. D. BIRD). *Research Laboratories, Parke, Davis & Co., Detroit, Mich.* Studies of the action of chloramphenicol on enzymatic systems have been conducted to determine if the action of the new antibiotic can be explained on the basis of an inhibitory effect on enzymes and enzyme systems. Members of the following classes of enzymes: esterases, carbohydrases, amidases, proteolytic enzymes, iron oxidases, copper oxidases, dehydrogenases and miscellaneous enzymes were subjected to the action of chloramphenicol. Three methods were used to evaluate these actions. The first method used was to test the action of the antibiotic on highly purified preparations of the isolated enzymes. The other 2 methods consisted of studies on the intact enzymes present in bacterial cells using the plate technique developed by Dufrenoy *et al.* and the broth culture technique. No indications of inhibitory action of chloramphenicol could be detected in any of the systems studied. The effect of various enzymes and enzymatic systems on chloramphenicol have also been studied to determine if the antibiotic can be destroyed by enzymatic conversion to an inactive form. Both isolated enzymatic systems and enzymes liberated into the culture media by intact organisms have been tested on the antibiotic. Results have shown that a partial breakdown of chloramphenicol is possible.

Phosphorus metabolism of molds. I. Uptake and transformation of orthophosphate by penicillium chrysogenum Q176. J. F. SNELL (by invitation) and W. H. PETERSON. *Univ. of Wisconsin, Madison, Wis.* Uptake of orthophosphate by *P. chrysogenum* Q176 in shaken flask and stirred jar fermentations on various media has been determined. Although there was considerable variation, the most rapid and complete uptake occurred in stirred jar fermentations on corn steep media. Mycelia varied in total phosphorus content (0.7%–5%, dry basis) depending upon aeration, age, media, etc. In a given fermentation, for example, the dried mycelium contained 3.1% at 3 days, reached a maximum of 4.7% at 6 days and fell to 2.1% at 8 days. On media containing a large excess of orthophosphate, higher amounts accumulated in the mycelium. From 30–60% of the total phosphorus was extracted by 4% trichloracetic acid in 2 hours at 0°. The proportion of labile phosphorus to total phosphorus in the extract (0–6%) was affected by medium, age of mycelium, method of extraction, etc. A typical extract of mycelium showed a $P_0:P_7:P_{>7}$ ratio of 25:6:69. Inorganic pyrophosphate and metaphosphate have been isolated from the extract, in confirmation of the work of Mann. Further components of this extract have been studied. No adenosine triphosphate has been obtained from the mycelium by procedures which gave good preparations from rabbit muscle in parallel experiments. Studies on the rate of turnover of the forms of phosphorus have been limited by inadequate fractionation procedures. Fractionation has not been successful by schemes proposed to date in the literature. Some fractionation has been achieved, however, and turnover studies utilizing P^{32} are in progress.

Aqueous dispersions of fat-soluble vitamins. ALBERT E. SOBEL, ABRAHAM ROSENBERG (by invitation), ROY GEDULDIG (by invitation), ELI ENGEL (by invitation), MICHAEL WEST (by invitation) and BENJAMIN KRAMER. *Dept. of Chemistry and Pediatrics, Jewish Hospital of Brooklyn, Brooklyn, N. Y.* Post-partum mother rats were fed vitamin A in aqueous dispersion or the same A in oily solution. The suckling rats of the aqueous group showed higher vitamin A stores. The same preparations had been previously fed nursing women. Distinctly higher rises of A were found in the milk of the aqueous group. Thus, one might conclude that with aqueous dispersions (without and with other vitamins: Vifort) there is an increased total secretion of milk vitamin A. The higher blood levels obtained with aqueous dispersions fulfill the theoretical requisites for greater diffusion to the site of

milk production. To produce controlled high levels and overcome absorption problems, conditions were developed for intravenous injection of aqueous dispersions of fat-soluble vitamins. No hemolysis occurs in whole blood when concentration of the dispersing agent is 0.1% or less. Bearing this in mind, aqueous dispersions of vitamins A and E were intravenously injected into rabbits with no pathologic effects. That aqueous dispersions may help in the absorption of other fat-soluble vitamins besides A was shown by studies with E. Higher absorption in normal adults occurred when aqueous dispersions of vitamin E were given orally as measured by vitamin E absorption curves.

Cystine and methionine content of portal blood plasma. JAMES D. SOLOMON (by invitation), and HAROLD D. WEST. *Dept. of Biochemistry, Meharry Medical College, Nashville, Tenn.* The cystine and methionine levels in the plasma of portal and peripheral blood were determined before and after administration of these amino acids to the dog. Since failure of cystine to rise in the peripheral blood plasma after feeding this amino acid has been demonstrated, it appeared that a study of its conten in the portal blood plasma might throw further light on the absorption of this amino acid. Cystine together with methionine was fed by gavage to the trachael-cannulated dog under ether anesthesia. Blood samples were taken from portal and femoral veins before feeding and at intervals of one and one-half and three hours. Cystine and methionine were determined in the plasma tungstate filtrates before and after hydrolysis by use of microbiological methods. The control samples from the portal and femoral (peripheral) vessels showed no increase in the microbiologically available methionine or cystine following hydrolysis of the tungstate filtrates. In samples taken after administration of these acids to the animal there was no significant rise in the level of the cystine in either case; but there was significant rise in the methionine level in portal blood accompanied by a much greater increase in its content in peripheral (femoral) blood. When these filtrates were hydrolyzed there was a considerable increase in cystine but the increase in methionine content of the portal blood plasma was not significant; in the peripheral blood filtrate hydrolysates there was no increase in the content of cystine or methionine.

Electrophoretic studies of the soluble proteins of rabbit liver. S. SOROF (by invitation) and P. P. COHEN. *Laby. of Physiological Chemistry, Univ. of Wisconsin, Madison, Wis.* Reproducible electrophoretic patterns have been obtained using high speed supernates of homogenates of perfused rabbit liver. All experiments were carried out in a cold room maintained at 4°C. Aliquots of whole liver, or homogenate, or supernate from the same rabbit liver were used for comparative electrophoretic experiments. The following factors were investigated with reference to their effects on the electrophoretic patterns: homogenization medium; pH stability; time stability; effect of freezing and thawing; and ultracentrifugation. From the large number of studies carried out, the following emerged as significant findings: *1*) the electrophoretic pattern of rabbit liver supernate consists of four principal components. The component in liver supernate with the mobility of rabbit serum albumin electrophoretically represents 4% of the soluble liver proteins. *2*) The soluble proteins of rabbit liver are unstable, unless special measures are taken. Certain of these alterations are reversible. *3*) Exposure to air causes changes principally in two components. Cysteine, thioglycollate and glutathione are each capable of reversing the aerobic changes in one of these two components. However, in order to reverse the aerobic changes in the other component, glutathione is required. Ascorbate is ineffective toward both components. *4*) In the case of first mentioned component, present evidence indicates that protein sulfhydryl groups are involved in a reversible reaction.

Composition of brain lipids in male and female rats. WARREN M. SPERRY, FLORENCE C. BRAND (by invitation), and MERRILL WEBB (by invitation). *Dept. of Biochemistry, New York State Psychiatric Inst., New York City.* Weil reported (*Growth*, 7: 257, 1943) that brains of female rats, 90–180 days old, contained more 'ether-soluble phospholipids (cephalin)' and less 'alcohol-soluble (lecithins), pyridine-soluble (galactolipids), and -insoluble lipids (sphnygomyelins)' than male brains. Such a sex difference should manifest itself in the P:choline ratio of the brain lipids. In a few preliminary measurements, reported previously (*Proc. Div. Biol. Chem., Am. Chem. Soc.*, 50C Sept., 1948), a small, but not statistically significant difference in this ratio was observed. However, further studies under more carefully controlled conditions the same average P-choline ratio was obtained in rats of both sexes. Following are the average values, with standard deviations, for various lipid constituents, determined in this investigation, expressed as percentage of fresh brain, from 125–156 days-old male and female rats, respectively: Choline, 0.462 ± 0.019, 0.469 ± 0.027; P, 0.245 ± 0.018, 0.248 ± 0.011; cholesterol, 1.89 ± 0.14, 1.89 ± 0.11; glycosphingosides, 2.47 ± 0.13, 2.45 ± 0.11. Average stoichometric ratios calculated from these data are: P:choline, 2.07 ± 0.15, 2.07 ± 0.19; cholesterol:choline, 1.28 ± 0.10, 1.27 ± 0.13; P:cholesterol, 1.62 ± 0.08, 1.64 ± 0.07; P:glycosphingosides, 2.63 ± 0.13, 2.68 ± 0.22. No support for the hypothesis that there is a sex difference in the lipid composition of the brain may be derived from these data. The findings will be com-

pared with values determined in the same way in rats of other ages.

Combined ultraspectrographic and chemical studies in cerebrospinal fluids (CSFs) in normal and pathologic conditions. M. SPIEGEL-ADOLF and H. T. WYCIS (by invitation). *Depts. of Colloid Chemistry and Neurosurgery, Temple Univ. Medical School and Hospital, Philadelphia, Penna.* Systematic ascorbic acid determinations in CSFs (*Federation Proc.* 7: No. 1) were made according to Robinson and Stotz. In 20 cases 2 ascorbic acid standards were used. The average error was 0.17 ± 1.83%. Ascorbic acid was determined simultaneously with the ultraspectrographic examinations. The extinction coefficients E_{CSF} at 265 mμ were corrected for the corresponding values of ascorbic acid E_A and proteins E_P. $D = E_{CSF}-(E_A + E_P)$ was determined in 60 cases of functional and organic neurological conditions. The lowest D values (0-0.14) were observed in psychoneurotics, old brain injuries, initial stages of brain affections. The highest D values (from 0.61–2.16) occurred in brain tumors, frequent grand mal attaques, spinal block, compression of the cord, recent cerebral concussion. Intermediate values were found in milder convulsive states, herniated discs, head traumata without unconsciousness and brain tumors of specified histological and/or anatomical properties. In an attempt to analyze the substances producing the D values micro-Kjeldahl determinations of the non-protein-nitrogen content were carried out in CSFs showing low and on samples showing high D values. The NPN values of the latter exceeded the supposedly normal values by an average of 50%. The above results seem to indicate that the selective absorption of pathologic CSFs at 265 μ is not explained by the ascorbic acid and protein content alone. Presence of nucleic acids and/or their cleavage products is suggested by the optical behavior. NPN determinations as well as the clinical findings support this interpretation.

Relative importance of plasmatic and vascular factors of hemostasis in the hemorrhagic diathesis of liver dysfunction. MARIO STEFANINI (introduced by A. J. QUICK). *Dept. of Internal Medicine, Univ. of Roma, Italy, and Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.* Of 200 patients with evident liver dysfunction, 30 (15%) presented spontaneous hemorrhages. The effectiveness of the hemostatic mechanism was studied in all, in an attempt to evaluate the relative importance of vascular and plasmatic factors of hemostasis in the pathogenesis of the hemorrhagic manifestations of these cases. Capillary fragility was variably increased in all patients. Plasma prothrombin activity was below 50% of normal in 53.3% of the cases, and below 20% in 23.3%. Plasma fibrinogen level varied from 90–356 mgr. % being, therefore, constantly above the critical level of 60 mgr. % established by Pinniger and Prunty. Changes in antithrombin activity of plasma were too inconsistent for definite conclusions. Therefore the diminution of capillary resistance was predominantly important in the pathogenesis of spontaneous hemorrhages in this series of liver patients. Furthermore, among the remaining 170 patients not presenting hemorrhages, capillary resistance was found diminished in 2 cases only, and prothrombin activity below 20% of normal in 9; so it appears that even a serious defect of the coagulation mechanism did not determine spontaneous hemorrhages when capillary resistance was normal. Hypoprothrombinemia and diminution of the capillary resistance found in liver disorders may present a common pathogenetic factor because of their association in about 50% of cases. As vitamin K normalizes the diminished capillary resistance of reversible liver dysfunction, this common factor might be represented by a defect of utilization of the vitamin by a diseased liver.

Effect of various adsorbants on the components of the prothrombin complex. MARIO STEFANINI (by invitation) and ARMAND J. QUICK. *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.* Oxalated plasma when treated with $Ca_3(PO_4)_2$, $Al(OH)_3$, $BaSO_4$, or $Mg(OH)_2$ will not clot on recalcification even in the presence of excess thromboplastin. These agents adsorb varying quantities of the components of the prothrombin complex and also some fibrinogen. By carefully regulating the quantity of adsorbant, a fairly quantitative separation especially of component B, the factor which diminishes in dicumarol poisoning, can be achieved. The best agent is $Ca_3(PO_4)_2$ since in the proper concentration (0.005M) it adsorbs component B completely, but removes only a trace of the labile factor and fibrinogen. The action of $BaSO_4$ is similar. Both of these adsorbants are unable to remove component B from citrated plasma, therefore no decrease in prothrombin activity occurs on mixing citrated plasma with either of these adsorbants. The prothrombin time of citrated plasma is prolonged to infinity by $Al(OH)_3$ and $Mg(OH)_2$. Citrate therefore does not block the adsorption of component B by these agents. Neither $Al(OH)_3$ nor $Mg(OH)_2$ are as selective as $Ca_3(PO_4)_2$ for they adsorb appreciable amounts of the labile factor, some fibrinogen, and probably other factors that are associated with coagulation. This explains why the prothrombin time curve constructed by diluting plasma with alumina treated plasma is similar to the curve using saline as diluent, whereas the curves obtained with $BaSO_4$ and $Ca_3(PO_4)_2$ treated plasmas as diluents are not.

Combination of wool protein with strong bases. JACINTO STEINHARDT and ETHEL M. ZAISER (by invitation). *Dept. of Chemistry, Massachusetts*

Inst. of Technology, Cambridge, Mass. Earlier work with 2 proteins, wool and egg albumen, has shown that their titration curves, obtained with different strong acids, differ widely in position on the *p*H axis. This effect is due to combination of the proteins with anions as well as hydrogen ions, and the differences between titration curves permit calculation of the relative affinities for protein of the anions. The effect of added salt on the titration is, in part, another manifestation of combination with anions. Since the effect of salt on the acid titration is exactly paralleled by its effect on titration with bases, these proteins must bind cations also. It is now shown that titration curves of wool at 0°C. with 10 strong monovalent bases also exhibit wide differences. As with anions, the affinity of cations for protein increases with increasing molecular dimensions. Among organic cations of the same weight, asymmetric ions produce much the larger shift. Unlike the effects of anions, which are uniform over the entire acid curve, the effect of cations is more marked on the same portion of the titration curve which is affected by formaldehyde, i.e., the part due to lysine. The maximum shift in position of this part of the titration curve, obtained with tetradecyltrimethyl ammonium hydroxide, exceeds two *p*H units. Marked effects on other portions of the curve are shown only by asymmetric ions larger than the hexyltrimethyl ammonium ion. Explanations of this distinction are suggested, and effects on the stability of the protein will be described.

Conversion of S^{35}-homolanthionine to S^{35}-cystine in the rat. JACOB A. STEKOL and KATHRYN WEISS (by invitation). *Lankenau Hospital Research Inst. and Inst. for Cancer Research, Philadelphia, Penna.* We reported that a mixture of isomers of homolanthionine is available to rats for growth purposes on a diet free of cystine but with minimal amounts of methionine. These results were interpreted as indicating the conversion of one or more isomers of homolanthionine to cystine *in vivo* (*J. Biol. Chem.* 175: 405, 1948). To secure a proof for the hypothesis we synthesized S^{35}-homolanthionine and fed it to young rats mixed with the diet (amino acid mixture diet) which was free of cystine but with 0.2% of methionine. After the rats grew for some time on the S^{35}-homolanthionine containing diet, the hair was clipped off, hydrolysed, and pure cystine was isolated and its radioactivity was measured. The product had a high radioactivity. These data establish beyond doubt the availability of the sulfur of homolanthionine for the synthesis of cystine *in vivo*.

Metabolism of hydroxyproline. MARJORIE R. STETTEN. *Dept. of Biological Chemistry, Harvard Medical School, and Division of Nutrition and Physiology, Public Health Research Inst. of The City of New York, New York City.* The amino acid hydroxyproline was synthesized in such a way as to contain about 30 atom % excess N^{15}. The synthetic mixture of 4 isomers was fractionated into the two DL forms. The DL(a) form, containing the naturally occurring *l*(−) hydroxyproline, was fed to 2 rats for 3 days and the distribution of N^{15} in the carcass, excreta, and a number of isolated amino acids was studied. The isotope analysis of the hydroxyproline isolated indicated that the dietary hydroxyproline was incorporated into the body proteins of the rat. The proline isolated contained only a trace of N^{15}, indicating that hydroxyproline is not converted to proline to any appreciable extent in the rat. This is in contrast to the previously observed extensive biological oxidation of proline to hydroxyproline. These findings are in accord with other indications that proline and hydroxyproline are not metabolically equivalent.

***d*-Aspartic oxidase of rabbit kidney and liver.** J. L. STILL and M. V. BUELL (introduced by D. E. GREEN). *Inst. for Enzyme Research, Univ. of Wisconsin, Madison, Wis.* Rabbit kidney and liver contain an oxidase which catalyzes specifically the oxidation by molecular oxygen of *d*-aspartate to oxaloacetate according to the equation: *d*-Aspartate + O_2 → oxaloacetate + H_2O_2. A stable preparation of this enzyme can be prepared by homogenizing the tissue, centrifuging off the insoluble residue, then removing the precipitate formed at *p*H 5.4 and finally making an acetone powder of the clear supernatant fluid. This preparation contains little if any of Krebs' *d*-amino acid oxidase. Unlike the *d*-amino acid oxidase the aspartic oxidase is insensitive to benzoic acid in M/300 concentration. The liver and kidney cyclophorase preparations at the 3rd residue stage contain significant amounts of the *d*-aspartic oxidase. Under appropriate conditions the complete oxidation of *d*-aspartate to CO_2 and H_2O can be accomplished. Fluoride and the presence of some member of the citric acid cycle are essential for the complete oxidation. Aspartate can be synthesized by incubating the cyclophorase system with oxaloacetate and NH_3 under aerobic conditions. The basic reaction appears to be: oxaloacetate + NH_3 → citrate + aspartate + CO_2. Under the aerobic conditions of the experiment citrate undergoes further oxidation. The oxidase does not oxidize *l*-aspartate, *d*-glutamate or other *d*-amino acids.

Metabolism of 16-ketoestrone and 16-keto-α-estradiol in man. BENJAMIN F. STIMMEL, ARTHUR GROLLMAN, and MAX N. HUFFMAN. *Rees-Stealy Medical Research Fund, Ltd., San Diego, Calif., and the Depts. of Pharmacology and Experimental Medicine and of Biochemistry, Southwestern Medical College, Dallas, Texas.* Colorimetric urinary estrogen excretion data on men after injection of 16-ketoestrone and 16-keto-α-estradiol indicate that man is capable of converting these compounds in part to estriol, but not to estrone and estradiol.

Zinc-hydrochloric acid hydrolysis of aliquots of the post-injection urine yields greatly augmented estrogen titers in the estradiol and estriol fractions of our (Stimmel. *J. Biol. Chem.* 162: 99, 1946) liquid chromatogram. There is evidence that 16-ketoestrone appears in the urine following injection with either of these compounds and that reduction of it to 16-ketoestradiol by zinc-hydrochloric acid hydrolysis of its conjugate in urine, accounts for the augmented estrogen titers obtained by this method of hydrolysis. These studies with exogenous estrogens therefore provide a working model in support of the hypothesis of Smith and Smith (*Endocrinology* 28: 740, 1941) that more highly oxidized estrogens than those presently known are excreted in the urine and that these may be converted to more active forms during hydrolysis by the addition of zinc dust.

Microbiological assay of vitamin B_{12} by Lactobacillus leichmannii. E. L. R. Stokstad, A. C. Dornbush (by invitation), A. L. Franklin (by invitation), C. E. Hoffmann (by invitation), B. L. Hutchings and T. N. Jukes. *Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y. Lactobacillus leichmannii* 313 (ATCC 7830) was reported to respond to the crystalline anti-pernicious-anemia factor (vitamin B_{12}). Further studies showed that thioglycollic acid increased the growth response produced by vitamin B_{12}. This was shown to be primarily due to protection of vitamin B_{12} against destruction during autoclaving of the samples with the medium. Protection was also accomplished by the addition to the medium of other reducing agents such as ascorbic acid or certain natural supplements that had been freed such as ascorbic acid or certain natural supplements that had been freed of thymidine and vitamin B_{12}. Similar results were obtained by adding the sample aseptically. Thioglycollic acid did not increase the response due to thymidine, presumably because thymidine is more stable and is not destroyed during autoclaving. *L. leichmannii* 313 was found to require a growth stimulant produced during autoclaving of the medium, as very little growth was obtained with a sterile-filtered or steamed medium even in the presence of vitamin B_{12}. This growth stimulation could alternatively be supplied by methyl glyoxal or thioglycollic acid, suggesting an effect due to a reduction in oxygen tension. Similar results were obtained with another strain, A.T.C.C. 4797, of *L. leichmannii*, which was found to grow more slowly and to have a lower requirement of vitamin B_{12} for half-maximum growth.

Non-participation of acetyl phosphate in the fixation of formate into pyruvate. Harold Strecker (by invitation) and Harland G. Wood. *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.* It has been previously demonstrated with *E. coli* extracts that synthetic acetyl phosphate was not an active intermediate in the dissimilation of pyruvate. The possibility that biologically produced acetyl phosphate might differ from the synthetic material was investigated in the following manner: C^{14} carbonyl labeled pyruvate was synthesized and incubated with cell free extracts of *E. coli*. Fermentation was allowed to proceed until 30–50% of the original pyruvate was determinable as acetyl phosphate by the Lipmann hydroxylamine method. The residual pyruvate was then diluted approximately tenfold with non-isotopic pyruvate, and C^{14} formate equivalent to the acetyl phosphate formed was also added for measurement of the extent of reversibility. An aliquot was withdrawn for analysis and degradation and the remainder was allowed to ferment. If the biologically formed high count acetyl phosphate was in dynamic equilibrium with pyruvate in accordance with the reaction $CH_3 \cdot CO \cdot COOH \rightleftarrows CH_3 \cdot CO \cdot OP(OH)_2 + HCOOH$, the final residual pyruvate should show an increase of specific activity in the carbonxyl group comparable to that of the carboxyl group as introduced from the formate. No such increase was obtained although C^{14} was introduced into the carboxyl group. It is therefore concluded that acetyl phosphate is not an intermediate in the formate exchange reaction. That exchange of formate may not occur through the phosphoroclastic reaction is indicated by experiments with aged enzyme. Pyruvate was not broken down as measured by acid production in bicarbonate yet exchange with formate occurred at the same rate as with a fresh preparation.

Effect of dihydroxyphenylalanine on enzymotic digestion. M. X. Sullivan and S. Stephen Schiaffino (by invitation). *Chemo-Medical Research Institute, Georgetown Univ., Washington, D. C.* In 1913, Torquato Torquati isolated a nitrogenous pyrocatechol complex from the germ of the broad bean (*Vicia faba*) and Guggenheim extracted a similar material from the broad bean pods and identified it as dihydroxyphenylalanine. Since then this compound has been found in a number of legumes. Some years ago we had occasion to study the lentil which had been found of poor nutritive value and we found that the lentil contained dihydroxyphenylalanine as determined colorimetrically. The same color reaction was positive with other legumes such as the Georgia velvet bean known to contain dihydroxyphenylalanine. Accordingly a study was made of the effect of dihydroxyphenylalanine on peptic and tryptic digestion of egg albumin. By the procedure described by Calvary (J. Biol. Chem. 102: 73, 1933) no inhibition of peptic digestion was found when dihydroxyphenylalanine was added at various levels to the digestion mixture, albumin, pepsin held at *p*H 2. In tryptic

digestion, however, dihydroxyphenylalanine has an inhibitory action. This was proved by estimating the amount of dye released from a dyed protein during 4–16 hours of digestion by trypsin and also by the amount of methionine released and estimated by the McCarthy, Sullivan colorimetric procedure. On estimating at different levels, it was found that 25 mg. of dihydroxyphenylalanine to 1 gm. of albumin had the same effect as 50 mg., a very marked inhibition. Even with 10 mg. and 5 mg. of 'dopa' there was 59 and 52% inhibition respectively in the amount of methionine liberated.

Reactions of hemoglobin with aliphatic and inorganic nitrate and nitrite esters. F. WILLIAM SUNDERMAN and HAROLD WOOSTER (by invitation). *Mellon Institute, Pittsburgh, Penna., and Cleveland Clinic, Cleveland, Ohio.* The quantitative aspects of methemoglobin formation have been studied following incubation at 38°C. of washed, laked human erythrocytes with solutions of potassium nitrate, butyl nitrate, diethyleneglycol dinitrate, nitroglycerine, potassium nitrite, and amyl nitrite. All of the nitrate compounds studied produce 2 molecules of methemoglobin per molecule of nitrate in accordance with the equation: $NO_3^- + 2Fe^{++}$ (hemoglobin) $\rightarrow NO_2^- + 2Fe^{+++}$ (methemoglobin). Our analyses reveal a disappearance of nitrate without a corresponding increase in nitrite. Hemoglobin solutions incubated with potassium nitrite for 5 hours at 38°C. yield 2 molecules of methemoglobin per molecule of nitrite; when incubated for 24 hours they yield more than 3 molecules of methemoglobin. If nitrites were to act directly as oxidizing agents, they would theoretically produce only 1 methemoglobin molecule per molecule of nitrite. The finding of higher ratios, therefore, suggests that nitrite probably first forms nitrate, which then reacts with hemoglobin to produce methemoglobin. Our studies indicate that amyl nitrite differs from other compounds studied in that it may either oxidize hemoglobin or reduce methemoglobin.

Activation of phosphoglucomutase. E. SUTHERLAND (by invitation), T. POSTERNAK (by invitation), and C. F. CORI. *Washington Univ., School of Medicine, Biological Chemistry Dept., St. Louis, Mo.* Various samples of glucose-1-phosphate (G-1-P), prepared enzymatically or chemically, were found to differ greatly in the rate of conversion to glucose-6-phosphate (G-6-P) by yeast or crystalline muscle phosphoglucomutase. Addition of active to inactive G-1-P raised the rate of conversion of the latter to that of the former. A possible explanation was the suggestion of Leloir and coworkers (*Arch. Biochem.* 19: 339, 1948) that glucose-1,6-diphosphate (G-1,6-P) was an activator in catalytic amounts according to the equation: G-1,6-P + G-1-P $\rightleftarrows$ G-6-P + G-1,6-P. G-1,6-P was synthesized by interaction of crystalline α-1-bromo-2,3,4-triacetyl-6-diphenylphosphonoglucose with silver phosphate, followed by catalytic hydrogenation to remove phenyl groups and partial hydrolysis with hydrochloric acid in methanol to remove acetyl and two hexosephosphate groups. The substance was isolated as the barium salt, more soluble in cold than in hot water, and gave correct analytical values for labile and stable phosphate, and for reducing power after hydrolysis. Cysteine (0.01M) was used to insure full activity of the enzyme. Cysteine could be replaced by heavy metal reagents such as diphenylthiocarbazone, 8-hydroxyquinoline, and histidine. Both the yeast and muscle enzyme were strongly activated by these reagents or protected by them when traces of zinc were added. The cysteine-activated enzyme did not convert G-1-P to G-6-P unless G-1,6-P was added in catalytic amounts, confirming Leloir's scheme. Half maximal activity of the enzyme was obtained when the concentration of the G-1,6-P was about 2×10^{-6} molar. A sample of G-1-P containing only 0.1% G-1,6-P will therefore be fully active in the usual reaction mixture contain 6×10^{-3} molar G-1-P.

Specificity of liver glucose-6-phosphatase. MARJORIE A. SWANSON (introduced by CAMILLO ARTOM). *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.* It has been known for some time that liver extracts hydrolyse hexosemonophosphates, but the number of enzymes concerned and their degree of specificity are uncertain. In the present investigation, extracts variously prepared from rat liver were found to liberate inorganic phosphate from glucose-1-phosphate, fructose-1,6-diphosphate, ATP, and glycerophosphate at a low rate, and from glucose-6-phosphate and fructose-6-phosphate at a much higher rate. Isoelectric precipitation at *p*H 5.5 increased the glucose-6-phosphatase activity/mg. of protein about threefold. Simultaneously the ability to split glucose-1-phosphate and fructose-1,6-diphosphate was lost, and the activity toward fructose-6-phosphate was diminished. The decrease in the rate of splitting fructose-6-phosphate paralleled the decrease in hexose isomerase activity, suggesting that its presence is necessary for the liberation of phosphate from fructose-6-phosphate. Thus it might logically be concluded that there is in the liver a specific glucose-6-phosphatase which, in the absence of phosphoglucomutase and hexose isomerase, hydrolyzes neither glucose-1-phosphate nor fructose-6-phosphate. Glycerophosphatase activity accompanied glucose-6-phosphatase (in the ratio of 1:5 to 1:6) through several attempts at purification. Presumptive evidence for the non-identity of these two enzymes was obtained on the basis of differential fluoride inhibition, lack of competitive inhibition, and different *p*H-activity curves.

Changes in the cholesterol concentration of r liver homogenates during incubation. LEON SWE (by invitation) and C. R. TREADWELL. *Dept.*

Biochemistry, George Washington Univ., School of Medicine, Washington, D. C. Normal rat livers were homogenized with 0.154M phosphate buffer of *p*H 7.2 and diluted to definite volume with buffer. All manipulations were performed at 0–3°C. Fifteen cc. aliquots were transferred to test tubes, mixed with 100 mg. of sodium glycocholate and placed in a shaker at 38°C. Duplicate 1 cc. samples were removed at zero hours and after various periods of incubation. The free and ester cholesterol were determined by a modification of the method of Hess (*Federation Proc.* 6: 260, 1947). During the first 2 to 4 hours the total cholesterol concentration decreased markedly; then decreased slowly until 6 hours had elapsed. With continued incubation the total cholesterol increased over that observed at 6 hours. After 8 to 12 hours the concentration approximated the zero hour value. Still longer incubation up to 36 hours usually gave values above the original level. The trend was toward a decrease in both free and ester cholesterol from 0 to 6 hours and an increase from 6 to 24. Studies of the influence of *p*H on these changes indicated that while the initial phase of decrease occurs over a range of 5.8 to 8.0, the increase in concentration occurs only from *p*H 6.8 to 7.2. Other experiments show that the observed changes in cholesterol concentration are not caused by microorganisms or volume changes. The effects of different temperatures and of the addition of possible precursors of cholesterol on these changes have been studied.

Isolation of a crystalline heat stable trypsin inhibitor from lima beans. HENRY TAUBER. *V. D. Research Laboratory, U. S. Public Health Service, U. S. Marine Hospital, Staten Island, N. Y.* A crude concentrate, containing at least two proteinase inhibitors, was prepared by a modification of Kunitz' (*J. Gen. Physiol.* 29: 149, 1946) procedure for the initial purification of crystalline soybean trypsin inhibitor. Ground lima beans were extracted with 0.25N H_2SO_4. The proteinase inhibitors were adsorbed on Bentonite, eluted with 10% aqueous solution of pyridine, precipitated by $(NH_4)_2SO_4$, dialyzed salt-free, and 3 volumes of acetone added for precipitation. From this precipitate a crystalline heat stable trypsin inhibitor was isolated as follows: the precipitate was dissolved in 1% NaCl solution. In order to remove impurities, a small amount of Bentonite was added and the mixture was shaken for 15 minutes. The clear filtrate was dialyzed for about 20 minutes against ethanol, applying occasional stirring. Small microscopic crystals formed. The solution was allowed to crystallize overnight at room temperature. The crystals were dissolved in 1% NaCl. Acetone was added till the mixture was slightly turbid. The solution was allowed to crystallize overnight at room temperature. Two recrystallizations of this material resulted in colorless octahedra of much larger size. This crystalline material is a globulin. It is precipitated by acetone, ethanol and trichloracetic acid. It does not dialyze through cellophane. It powerfully inhibits crystalline trypsin. Proteolysis and milk clotting by chymotrypsin are moderately effected. The proteolytic and milk-clotting action of pepsin are not inhibited. This trypsin inhibitor somewhat resembles Kunitz' crystalline soybean trypsin inhibitor. In contrast to his inhibitor, however, the crystalline lima bean inhibitor is exceptionally heat stable. The soybean inhibitor is destroyed on short boiling.

Molecular weight of human fetal hemoglobin. JOHN FULLER TAYLOR and RICHARD L. SWARM (by invitation). *Washington Univ. School of Medicine, Dept. of Biological Chemistry, St. Louis, Mo.* We have measured the sedimentation and diffusion constants of fetal oxyhemoglobin (HbO_2) prepared from cord blood taken from normal full-term infants. The erythrocytes were washed with saline solution and laked with toluene. Adult HbO_2 solutions were similarly prepared from bank blood. Sedimentation velocity was measured in a 'Spinco' analytical ultracentrifuge at 20 to 30°. Diffusion was followed in a Claesson cell at 2°. Results have been corrected to water at 20°. We observed for fetal HbO_2 an $S_{20,w}$ of 3.98 at 0.9% protein, and 4.01 at 0.5%, and for adult HbO_2 an $S_{20,w}$ of 4.00 and 3.99 respectively. Essentially identical S values were obtained for fetal HbO_2 and HbCO in dilute phosphate buffer. M. A. Andersch, D. A. Wilson, and M. L. Menten (*J. Biol. Chem.* 153: 301, 1944) had reported values of 2.6 to 3.0 for 0.5% fetal hemoglobin. An average diffusion constant, $D_{20,w}$, of 6.6 cm.^{2} sec^{-1} was obtained for 1% fetal HbO_2 in 1% NaCl. D values of 6.3 to 6.9 for adult hemoglobin have been reported in the literature. The identity in the sedimentation constants and the agreement in the diffusion constants of fetal and adult HbO_2 indicate that the hemoglobins from either source have the same molecular weight under the conditions of our experiments.

Bound coenzymes of the cyclophorase complex of rabbit kidney and liver. L. J. TEPLY (introduced by D. E. GREEN). *Institute for Enzyme Research, Univ. of Wisconsin, Madison, Wis.* The cyclophorase gel of rabbit kidney or liver at the third residue stage (exhaustively washed with dilute salt solution) contains considerable amounts of the following coenzymes in bound form: adenine nucleotides, pyridine nucleotides, acetylation coenzymes, flavin dinucleotide, pyridoxal phosphate and diphosphothiamine. It also contains appreciable amounts of bound biotin and folic acid. The adenine nucleotides exist in the form of AMP, ADP, and ATP. By various devices AMP can be converted largely to ATP and vice versa. During storage of the enzyme at 0° for several days or at 38° for some minutes the adenine nucleotide level declines. The pyridine

nucleotides are a mixture of CO_I and CO_{II} in approximately equal amount. The relative proportion of these two coenzymes remains fairly constant under a variety of conditions. With aging of the enzyme there is a steady decline of the level of pyridine nucleotides. The bulk of the acetylation coenzyme of rabbit liver and kidney appears to be bound up with the protein of the cyclophorase complex. All the above coenzymes are released from the cyclophorase gel in varying degrees by treatment with boiling water or 5% trichloracetic acid. A crude barium salt fraction prepared from the neutralized trichloracetic acid extract contains mainly pyridine nucleotides, flavin dinucleotide, the acetylation coenzyme, adenine nucleotides and inorganic phosphate.

Studies of growth factors in liver extract using paper partition chromatography. GARSON H. TISHKOFF, H. T. PEELER, ALEJANDRO ZAFFARONI, HENRY TESLUK, and L. C. NORRIS (introduced by ELMER H. STOTZ). *Depts. of Pathology and Biochemistry, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y., and School of Nutrition, Cornell Univ., Ithaca, N. Y.* Recent investigations by Daniel, Peeler, Norris, and Scott (*J. Biol. Chem.*, in press) have demonstrated the presence of two unidentified factors in purified liver extract which are distinct from strepogenin but required by Lactobacillus casei for growth. These investigators have also demonstrated that a correlation exists between the antipernicious anemia activity of the extract and the growth response of Lactobacillus casei to one of these factors (APA factor) in the presence of an excess of the second factor ('whey' factor). In order to establish the nature of these factors, and as a possible method for their isolation in pure form, liver extract was fractionated using paper partition chromatographic technique and the individual fractions assayed for the APA factor and 'whey' factor.

Preparation of hyaluronate from some mammalian sources. SIBYLLE TOLKSDORF (by invitation), JAMES W. CASSIDY (by invitation), CAROL BONSAL (by invitation) and D. ROY MCCULLAGH. *Dept. of Biochemistry, Schering Corporation, Bloomfield, N. J.* Human umbilical cords, bovine synovial fluid, bovine vitreous humor and hog belly skin have been processed by various methods in investigating the production of hyaluronic acid. Modifications of the Rogers method (*Biochem. J.* 39: 1945) have been employed and in general his technique has been used successfully except in the case of hog belly skin. However, it has been found that certain substances which interfere with the assay of testicular hyaluronidase are frequently present in such hyaluronate preparations. We have found that these impurities can be removed by washing the crude mucin clot with 0.2% acetic acid before separating the proteins by precipitation with chloroform. A simpler method has been employed for the production of hyaluronate from umbilical cords for assay purposes. The macerated cords were heated with water to 80°C. to precipitate proteins. Impurities were removed by adsorption on bentonite or other agents. The hyaluronates form a white powder when precipitated with acetone and dried with acetone and ether. No success has been attained in applying this procedure to other starting materials. Elementary chemical analysis would suggest that the polysaccharide from all these sources is essentially the same. The characteristics of the hyaluronate molecule which govern its viscosity must be due to different aspects of the molecular structure than those which control its capacity to cause turbidity with acidified proteins. This is attested to by the fact that preparations of similar purity show no quantitative relationship between the two phenomena.

Biochemical studies on the livers of chicks receiving graded levels of pteroylglutamic acid. JOHN R. TOTTER, WILLIAM MARTINDALE (by invitation), MARION MCKEE (by invitation), CECILIA K. KEITH (by invitation), and PAUL L. DAY. *Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock, Ark.* Groups of day-old White Leghorn chicks were given a purified diet (KEITH ET AL. *J. Biol. Chem.* 176: 1095, 1948) supplemented with 0, 5, 10, 20, 40, 80, 200, and 1000 μg. of pteroylglutamic acid (PGA) per 100 grams. An additional control group received a commercial starter diet. At the end of 4 weeks the birds were killed. The livers were analyzed for *1*) PGA, using *Streptococcus faecalis* and conjugases from hog kidney and chicken pancreas, *2*) conjugase, *3*) desoxypentose nucleic acid and *4*) pentose nucleic acid. The PGA levels in the livers indicated that storage of excess vitamin did not occur until the dietary level exceeded 40 μg/100 gm. The liver PGA freed by autolysis was approximately the same as that determined with conjugases from hog kidney or chicken pancreas. The conjugase levels of the livers appeared to be unrelated to either the dietary level of PGA or the nucleic acid content of the livers. Desoxypentose nucleic acid was found to be somewhat low in the chicks receiving no dietary PGA but the differences among all other groups were of doubtful significance.

Cholesterol esterase of pancreatin. C. R. TREADWELL, R. S. YAMAMOTO (by invitation), and NORMAN P. GOLDSTEIN (by invitation). *Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington, D. C.* Preliminary to an investigation of the significance of cholesterol and its esters we have attempted to evaluate the optimum conditions for demonstrating the activity of the enzyme. Commercial pancreatin was used as a source of cholesterol esterase. In most of the previous work natural materials, such as serum, have

been used to supply the substrate. In these experiments synthetic cholesterol oleate has been used as the substrate. Technical difficulties were encountered in the preparation of a stable emulsion of the ester. When various emulsifying agents were tested, singly and in combination, the most efficient found was phosphate buffer containing albumin and bile salts. When cholesterol oleate was emulsified in this mixture and the emulsion incubated at 38°C. with shaking, the solution was homogeneous in respect to the ester for periods up to 48 hours as shown by analysis of duplicate aliquots. When 1 cc. of a 20% suspension of pancreatin in 50% glycerol and 14 cc. of the substrate mixture were incubated for 24 hours, 45% hydrolysis was obtained. The rate of hydrolysis was constant for 24 hours. The optimum *p*H was found to be approximately 7. Heat inactivation experiments indicated that the enzyme was completely inactivated by heating at 65°C. for 1 hour. The degree of hydrolysis was proportional to the concentration of the enzyme.

Quantitative aspects of coprecipitation in the purification of substances isolated in tracer studies. SIDNEY UDENFRIEND (by invitation), ALBERT S. KESTON (by invitation), NORA LEVITAS (by invitation) and MILTON LEVY. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.* In the isotopic derivative method we encountered, in several instances, considerable difficulties in the elimination of impurities by crystallization. The basis of the difficulty was a distribution of the impurity between crystals and solution as though between immiscible solvents and may be formulated $K = I_s/C_s/I_c/C_c$ where I and C are the amounts of impurity and carrier in supernatant (subscript s) and crystals (subscript c). The amount of purification achieved in a single crystallisation is greater the larger is K and the greater the loss of carrier in the supernatant. Addition of the non-isotopic analogue of the impurity to dilute its activity has no advantage as long as the above formulation holds and it has been found to hold in instances from an impurity level of 0.0001% to 5%. Crystallization of DL leucine containing S-35 methionine gave K 2.5. For alanine with glycine as the impurity K = 1.5. Practically the same constants are found for the same amino acids as pipsyl derivatives. Thus it is not necessarily advantageous to prepare different derivatives in the course of purification. As an example of the import of this phenomenon only by 200 recrystallizations, sacrificing 5% of the remainder at each, would a system with K = 1.5 reach a level of 1% of the original impurity. Coprecipitation must be considered in evaluating any 'constant isotope concentration' attained by crystallization. Chromatographic and countercurrent solvent extractions techniques are more dependable in this respect.

Action of streptomycin in tissue homogenates. W. W. UMBREIT and N. E. TONHAZY (by invitation). *Merck Institute for Therapeutic Research, Rahway, N. J.* Previous reports have demonstrated that one of the actions of streptomycin is to inhibit the condensation between oxalacetate and pyruvate in susceptible strains of *Escherichia coli*. Inasmuch as this is an important reaction in animal cells, the question arises as to why streptomycin is able to enter the animal body, kill or inhibit the susceptible bacteria contained in it, without comparable harm to the animal. The condensation of oxalacetate and pyruvate is largely localized in the mitochondrial fractions, whose activity may be preserved by homogenizing in isotonic KCl or isotonic sucrose. Using isotonic homogenates from rat liver and rat kidney, it was demonstrated that streptomycin in concentrations of 30–60 γ/ml. had no effect upon the oxalacetate-pyruvate reaction. However, when the concentration was increased to 300 γ/ml. inhibition was frequently obtained. Inhibition of this reaction could be demonstrated for low levels of streptomycin if the homogenate and the streptomycin were incubated in the cold for two hours. The circumstances of these experiments are such as to suggest that streptomycin will inhibit the oxalacetate-pyruvate condensation in the animal as well as in the bacteria but that in the animal there is a permeability barrier at the cell wall and at the mitochondria which prevents the streptomycin from reaching the enzymes in the animal cell concerned with catalyzing this reaction.

Conversion of isotopic pyruvate to oxalacetate by pigeon liver. M. F. UTTER and MARY T. CHENOWETH (by invitation). *Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* Although it has been assumed that the formation of oxalacetate in the Wood-Werkman reaction is accomplished by the carboxylation of pyruvate, previous studies have dealt only with CO_2 without identification of the other component. In the present work, the incorporation of labeled pyruvate into oxalacetate has been compared with the incorporation of C^*O_2 to determine the nature of the immediate precursor of oxalacetate. Carboxyl labeled pyruvate was synthesized from acetylbromide and $Cu_2(C^*N)_2$ via pyruvonitrile and pyruvamide. Oxalacetate was decarboxylated by a pigeon liver preparation in the presence of pyruvate and C^*N_2 and the residual oxalacetate separated and purified as the mercurous salt. The oxalacetate was analyzed for isotope content in the two carboxyl groups since one group originates from CO_2 and the other from the pyruvate carboxyl. Results indicate that the fixation of pyruvate in oxalacetate occurs with certain enzymatic preparations but is not correlated with the fixation of CO_2. Some enzymatic preparations that fix CO_2 very rapidly are inactive on pyruvate. In contrast to the marked stimulation of CO_2 fixation caused by ATP, when

pyruvate fixation occurs it is relatively unaffected by ATP. The foregoing findings lead to the tentative conclusion that pyruvate is not the immediate precursor of oxalacetate. Preliminary results show that labeled pyruvamide can be substituted for pyruvic acid in the reaction mixture and is also incorporated in a compound resembling oxalacetate. The mechanism is under investigation.

Isotopic studies of the biosynthesis of nucleic acid components. II. allantoin. JEROME D. VALENTINE (by invitation), SAMUEL GURIN and D. WRIGHT WILSON. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.* It has been established that the carboxyl carbon of glycine is a source of carbon 4 in the uric acid excreted by the pigeon. In order to determine what role glycine plays in the biosynthesis of purines in mammals, glycine labeled in the carboxyl position with C^{14} was mixed with a stock diet and fed to young albino rats for a period of 10 days. From pooled 24-hour collections of urine, allantoin was isolated, purified and analyzed. All samples of allantoin were found to be radioactive, the specific radioactivity increasing gradually to a maximum on the 10th day. By degradation of allantoin to glyoxylic acid and urea the C^{14} was found in the glyoxylic acid fraction, corresponding to carbons 4 and 5 of the allantoin molecule. Allantoin isolated after giving carboxyl labeled sodium adetate intraperitoneally to 5 rats over a 5-day period was found to be very weakly radioactive. It would appear that this compound is either not directly utilized as such for purine synthesis or that it is directed into other metabolic pathways. These results are of interest in view of the fact that much of the carboxyl carbon of acetate is incorporated into carbons 2 and 8 in the uric acid excreted by the pigeon.

Inactivation of stilbestrol in alkali. RAYMOND VANDERLINDE (by invitation) and W. W. WESTERFELD. *Dept. of Biochemistry, Syracuse Univ. College of Medicine, Syracuse, N. Y.* The inactivation of stilbestrol that occurs on standing in an alkaline solution results from a dehydrogenation of the stilbestrol to isodienestrol and a subsequent ring closure to indenestrol. Both of these products have been isolated from reaction mixtures. A nearly quantitative conversion to indenestrol was obtained when 0.8 mg of stilbestrol per cc. of 0.8N NaOH stood at room temperature for 30 days. Isodienestrol and small amounts of unchanged stilbestrol were the principal products obtained when 1 mg. of stilbestrol per cc. of 0.02N NaOH stood at room temperature for 14 days. Isodienestrol is approximately 1/700 as active estrogenically as stilbestrol; indenestrol is at least 1/10 as active as stilbestrol. The relative decrease in estrogenic potency of the original stilbestrol solution will obviously depend upon the proportions of the three main constituents in the final solution, and this varies with the conditions of inactivation.

Antigenic transformation in paramecium aurelia under the influence of proteolytic enzymes. W. J. VAN WAGTENDONK. *Dept. of Zoology, Indiana Univ., Bloomington, Ind.* Within stock 51 of variety 4 of *Paramecium aurelia* 6 antigenically diverse strains have arisen (strains A, B, C, D, E and G). Each of these 6 types is inherited under standard conditions of culture. Differences in antigenic type do not depend on differences in genes but on cytoplasmic differences. These cytoplasmic factors are subject to environmental influences. Exposure to dilute antiserum, high or low temperature, brings about a transformation in antigenic type to any one of the possible types in this stock. The new antigenic type is inherited true to type, as long as the cultures are grown under standard conditions (Sonneborn. *Scientific Monthly* 67: 154, 1948). Five strains of *P. aurelia* (stock 51, variety 4) have been exposed to the action of trypsin and chymotrypsin at 27° for 12 and 24 hours. After exposure single isolations were grown for 8 fissions at 20°, 27° and 32°, and the antigenic type of the resulting clones determined. Under these conditions, strains 51A, 51B and 51G very rarely transformed into another type. Types 51C, 51D and 51E gave rise to a large variety in types. The susceptibility to trypsin and chymotrypsin of the latter three types is correlated with their instability under other external conditions.

Influence of biliary obstruction upon liver protein regeneration. HARRY M. VARS, COLIN C. FERGUSON (by invitation) and CHARLES S. ROGERS (by invitation). *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.* Groups of male Wistar rats, previously protein-depleted for a period of 14 days, were subjected to partial hepatectomy (70%) and to simultaneous high ligation and division of the common bile duct. Postoperatively these animals were fed a 10% protein, low fat diet and were then killed on the 2d, 4th, eighth and 14th days. Control groups, only partially hepatectomized, were pair-fed with the above rats. Total nitrogen balances were determined. Following death the amounts of new liver protein were measured, together with liver mass (wet and dry), glycogen and total lipid. Histological examination of all livers was made. At all periods postoperatively it was found that the biliary-obstructed rats not only had livers of greater mass but livers containing greater amounts of new liver protein than did the controls. Undoubtedly a portion of this protein increment is ductal in origin, and some may be present in the increased fluid content of the obstructed livers as plasma or lymph. However, estimation of the ductal proliferation as seen on histological examination and estimation of the protein associated with the

increased fluid indicate that liver protein regeneration occurred in the biliary-obstructed rat to a degree almost equal to that in the unobstructed pair-fed animal. Counts of parenchymal cell mitoses in both groups were approximately equal.

Rat liver fructokinase. CARL S. VESTLING, URSULA IRISH (by invitation), AUGUSTA K. HIRSCH (by invitation) and NORMAN H. GRANT (by invitation). *Division of Biochemistry, W. A. Noyes Laboratory of Chemistry, Univ. of Illinois, Urbana, Ill.* A study of the phosphorylation of glucose and fructose by rat liver homogenates has been carried out. It has been found that systems which phosphorylate fructose very effectively are quite inert to glucose. Addition of numerous materials (DPN, HDP, phosphocreatine, insulin, various phosphatase inhibitors and others) fails to allow phosphorylation of glucose at a rate at all comparable to that encountered with fructose. The Nelson procedure (*J. Biol. Chem.* 153: 375, 1944) and that of Roe (Browne and Zerban. *Sugar Analysis*, p. 961, 1941) have been employed in studying sugar disappearance. Slightly hypertonic phosphate buffer homogenates of livers from fasted rats have been used in this work. The complete systems contain ATP, $MgCl_2$, α-ketoglutarate or glutamate, substrate and homogenate. The effect of NaF has been studied. In certain experiments liver slices have been substituted for homogenates with essentially similar results. The oxidation of α-ketoglutarate or glutamate by these systems greatly improves the phosphorylating capacity. When glucose-1-phosphate, glucose-6-phosphate, or fructose-6-phosphate is incubated in rat liver systems, about one-fifth reappears as 'apparent glucose' and none as fructose. When a beef brain hexokinase preparation, which will phosphorylate both glucose and fructose, is added to a rat liver system, the combined system phosphorylates both glucose and fructose effectively. The combined system phosphorylates about twice as much glucose as the brain hexokinase alone. Boiling the brain hexokinase extract abolishes this effect.

In vitro utilization of C^{14} labeled acetate and pyruvate by rat diaphragm muscle. CLAUDE A. VILLEE, OLOF H. PEARSON (by invitation) and A. BAIRD HASTINGS. *Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Diaphragm muscle from normal, diabetic and adrenalectomized rats was incubated, using the technique of Gemmill, in a medium containing 0.04M sodium phosphate, 0.005M $MgCl_2$, 0.08M NaCl, and either carboxyl labeled C^{14} acetate or carbonyl labeled C^{14} pyruvate, 10 mM/l., initial *p*H, 6.8. A half diaphragm was placed in each Warburg vessel; to one of each pair of vessels 0.5 U insulin/ml. medium was added. The diaphragms were incubated for 2 hours in an atmosphere of oxygen. The CO_2 collected in the center well was precipitated as $BaCO_3$, plated and counted with an end window Geiger tube. Samples of the media before and after incubation were analyzed for pyruvate and lactate, and the amount of glycogen in the diaphragm before and after incubation was estimated. The metabolism of acetate to CO_2 in diabetic diaphragm was decreased to 35% of normal and this decrease was unaffected by the addition of insulin. Insulin had no effect on the metabolism of acetate to CO_2 in normal diaphragm. Experiments on the effect of adrenalectomy and on the metabolism of labeled pyruvate are underway and will also be reported.

Anesthesia with methylal in mice and dogs. ROBERT W. VIRTUE. *Division of Anesthesiology, Dept. of Surgery, State Univ. of Iowa, Iowa City, Iowa.* Exposure of mice to concentrations of 7% methylal ($CH_3—O—CH_2—O—CH_3$) in air was followed within 10 minutes by loss of reflexes producing a condition compatible with surgical anesthesia. Increasing the concentration to 17.5% methylal produced respiratory arrest. The anesthetic index was therefore 2.5. The corresponding index for ethyl ether was 2.0. Twenty-five% methylal in 0.9% sodium chloride solution was injected intravenously into dogs. Surgical anesthesia was produced with blood concentrations of methylal between 109 and 235 mg. %. After injection of sufficient methylal to produce respiratory arrest the blood methylal values varied between 265 and 453 mg. %, and one hour later the blood methylal values ranged between 99 and 295 mg. %. As has been consistently observed during ether anesthesia, concentrations of blood glucose rose considerably during methylal anesthesia, the carbon dioxide combining power diminished, and the blood urea values increased slightly. Recovery of both mice and dogs from anesthesia with methylal was much slower than from anesthesia with ether, probably due to slower elimination of methylal because of its greater solubility in water.

Chromatographic separation of ribonucleotides and its application to the study of ribonucleic acid structure. ERNST VISCHER (by invitation), BORIS MAGASANIK (by invitation), and ERWIN CHARGAFF. *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York.* The ribonucleotides derived from yeast ribonucleic acid, viz. adenylic, guanylic and cytidylic acids, can be separated from each other and also from muscle adenylic acid by chromatography on filter paper with isobutyric acid (saturated with water) as the solvent in a suitably maintained NH_3 atmosphere. The separated components are fixed on the paper as the uranyl salts and demonstrated by the conversion of the latter to brown uranyl ferrocyanide. Parallel undeveloped chromatograms serve for the extraction of the adsorbates with M phosphate buffer of *p*H 7.1 and the spectrophotometric estimation of the separated nucleotides by proce-

dures similar to the recently described method for purines and pyrimidines (Visscher and Chargaff. *J. Biol. Chem.* 176: 703, 1948). The recoveries ranged from 90 to 100%. If the distance of the yeast adenylic acid adsorbate from the starting point is taken as 100, muscle adenylic acid is 75, cytidylic acid 60, and guanylic acid 40. Alkaline hydrolysates of yeast ribonucleic acid exhibited 3 zones of adsorption which in their positions on the chromatogram and in their absorption spectra corresponded to guanylic, cytidylic and adenylic acids, respectively, accounting for a total of 75–85% of the nucleic acid used. The quantitative distribution of the nucleotides thus determined following the degradation by various means of ribonucleic acids of yeast and of mammalian tissue will be discussed with particular emphasis on the bearing of these studies on the problem of nucleic acid structure.

Function of the system, glutamic acid-glutamine, in brain metabolism. HEINRICH WAELSCH, PAULA SCHWERIN (by invitation) and S. P. BESSMAN (by invitation). *Depts. of Biochemistry, N. Y. State Psychiatric Institute and Columbia Univ., New York City.* The protein-free filtrate of brain (rat, mouse, rabbit, calf) contains about 160 mg. glutamic acid and 60 mg. glutamine/100 gm. tissue. The concentration of free glutamic acid in brain is higher than in any other organ analyzed (muscle, liver, kidney, blood). After intravenous injection of large amounts of glutamic acid or of glutamine into rats and mice no increase in the glutamic acid content of the brain was found while the entrance of glutamine into the brain could not be excluded with certainty. Glutamine appears to enter the liver with greater ease than does the parent amino acid. The data suggest that brain glutamic acid may be derived from glucose through the tricarboxylic acid cycle. At present, however, the possibility still exists that this amino acid originates at least in part from circulating glutamine. The function of the system glutamic acid-glutamine is discussed as one of the metabolic buffers of the tricarboxylic acid cycle. Under physiological conditions the large concentration of glutamic acid in the brain assures an optimal functional rate of the cycle at the ketoglutaric acid step. Glutamic acid may furthermore act as a respiratory substrate under conditions of impaired glucose supply.

Influence of lipotropic compounds on the lipid and protein fractions of blood plasma from children with nephrotic syndrome. CHI CHE WANG, H. H. BOYLE and S. FREEMAN. *Children's Memorial Hospital & Northwestern Univ. Medical School, Chicago, Ill.* A total of 11 children with nephrotic syndrome and lipemia were treated during varying periods with one or more of the following compounds: Methionine, inositol, choline. In some cases an effect was demonstrated in the blood lipid values. In one case all fractions but phospholipids definitely decreased following the ingestion of 3 gm. of inositol for 11 weeks and 5 days. On the other hand 4 of the 5 patients receiving inositol alone showed no consistency in the lipid values during inositol intake. One out of 5 patients showed a definite decrease in lipid values during the administration of choline alone. Two children who received a combination of 3 gm. of inositol and 2 gm. of choline for 9 days in the case of one patient and for 14 weeks 4 days in another patient showed a definite lowering of all the lipid fractions. Four out of 5 children receiving 5 gm. of methionine showed a lowering of the lipid fractions with some fluctuations. In the fifth child there was a slight increase in neutral fat. The lipotropic compounds did not produce a definite change in the protein fractions of blood plasma of these patients.

Determination of pyrimidines by the isotope derivative method. ROBERT C. WARNER. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.* Conditions have been established for the formation of derivatives of uracil and thymine with pipsyl (para-iodo-phenyl sulfonyl) chloride. The reaction proceeds at room temperature in 75% acetone solution buffered with bicarbonate to give a 94% yield of the mono pipsyl ester. With I^{131} in the derivatizing reagent, this reaction has been made the basis of a quantitative determination of uracil and thymine by the same method that has been applied to amino acids by Keston, Udenfriend and Cannan (*J. Am. Chem. Soc.*, in press). The pyrimidine solution is derivatized with isotopic reagent of known specific activity. A large excess of the unlabelled derivative of the desired constituent is added as carrier. A sample is purified to constant specific activity from which the quantity of the constituent originally present may be calculated. Purification is accomplished by means of repeated recrystallization with norite treatment and countercurrent distribution. The latter method makes it possible to analyze for both uracil and thymine on the same reaction mixture after adding carriers for both. Recoveries from mixtures and nucleic acid hydrolysates indicate an overall accuracy of 1–2% on quantities of 10 μg. Cytosine can be determined after conversion to uracil. Data on the pyrimidine content and rate of hydrolysis of nucleic acids have been obtained by this method. The indicator technique (*Keston, Udenfriend and Levy. J. Am. Chem. Soc.* 69: 3151, 1941) using both isotopes I^{131} and S^{35} and employing paper strip chromatography can also be applied to 0.1 μg. quantities of pyrimidine.

In vitro metabolism of testosterone and its metabolites by kidney tissue. CHARLES DONALD WEST (by invitation) and LEO T. SAMUELS. *Dept. of Biochemistry, Univ. of Utah, Salt Lake City, Utah.* This investigation was undertaken to determine whether or not the kidney is involved in the

metabolism of testosterone. When testosterone was incubated with dog or rabbit kidney mince, the testosterone was destroyed enzymatically and 17-ketosteroids were formed. This destruction was accomplished by at least two enzyme systems. One has DPN for its coenzyme and is concerned in the oxidation of the hydroxyl group on C-17 to the ketone. The other enzyme system is much less active and is concerned in the saturation of the α-β unsaturated bond in the A ring. When androstenedione was incubated with rabbit kidney mince, the 17-ketone group was destroyed while the α-β unsaturation in the A ring was left intact. The addition of citrate greatly accelerated this reaction. Similarly, the 17-ketone group of androsterone was destroyed when it was incubated with rabbit kidney mince. This destruction was also catalyzed by the addition of citrate. No α-β unsaturated steroids were formed from the androsterone. The relation of these enzymes to those in the liver will be discussed.

Dietary effects on liver xanthine oxidase. W. W. WESTERFELD and DAN A. RICHERT (by invitation). *Dept. of Biochemistry, Syracuse Univ. College of Medicine, Syracuse, N. Y.* Rats were born without detectable liver xanthine oxidase activity. At 21 days of age the average activity was 720 units (Cmm O_2/gm. dry liver/hr.). Adult rats on dog chow averaged 1550 U. Weanling rats were brought to 1535 and 1385 U respectively by feeding dog chow (21% protein) or mineralized whole milk for 2 weeks. Feeding purified diets containing 21% protein (casein, egg albumin, peanut protein) maintained the liver xanthine oxidase activity at approximately the weanling level (600–900 U) for about 6 weeks, but gave values of 1000 to 1200 U in 8 to 10 weeks. Increasing the protein content of the diet to 25 and 30% by the addition of peanut meal gave only 900 to 1000 U of liver xanthine oxidase after 6 weeks. Supplementing the 21% purified casein diet with 10% dry liver gave 1290 U of activity in 2 weeks; supplementing the diet with raw cream was similarly effective with some samples of cream but not with all. A diet containing 21% crude casein gave 1260 U of activity in 4 weeks. Supplementing the purified casein diet for 2 weeks with a mixture of Cu, Zn, Mn, Co, and I or with a mixture of flavine, adenine, and ribose or with a mixture of biotin, inositol, p-aminobenzoic acid, pteroylglutamic acid, rutin and ergostanyl acetate did not significantly increase the liver xanthine oxidase above the control levels. A concentrate of vitamin B_{12} was also ineffective.

Progesterone and testosterone reversal of diethylstilbestrol inhibition of succinoxidase of tumor mitochondrial elements. KENT WIGHT (by invitation) and DEAN BURK. *National Cancer Institute, National Institutes of Health, Bethesda, Md.* It has been found that certain hormones act competitively on the succinoxidase system of the mitochondrial elements of the S91 and Harding-Passey melanomas grown in dba and C mice respectively. The mitochondrial elements (microscopically visible cytoplasmic particulates, mostly melanized; see Woods *et al. J. Nat. Cancer Inst.* Feb. 1949) were obtained by centrifugation at 10°C. of saline, glass wool-filtered tumor homogenates at 10,000 *g* for 30 minutes, after preliminary centrifugation at 25 *g* for 10 minutes to eliminate nuclei and heavy phagocytized cell material. Oxygen consumption was measured manometrically after addition of 0.05M phosphate (*p*H 7), 0.00001M cytochrome C, and 0.02M succinate. Diethylstilbestrol, at a concentration of ~135 γ/ml. (≈ 0.0005M), inhibited particulate oxygen consumption 60 to 75%. This inhibition was markedly reduced or entirely eliminated by the simultaneous addition of ~125 γ/ml. (≈ 0.0002M) progesterone or testosterone. Progesterone alone had no effect on oxygen consumption. The S91 and Harding-Passey tumor particulates behaved similarly except that the normal Q_{O_2} values (ml.^{3} O_2 consumed/mg/dry wt/hour) were about 5 times as great in the case of the former (Q_{O_2} of 10–20 compared to 2–5), and that testosterone alone sometimes inhibited the latter. Essentially no succinoxidase activity was shown by the initial nuclei fractions or the final supernatants. Diethylstilbestrol also markedly inhibited or eliminated oxygen consumption by mitochondrial elements from non-melanoma tumors such as C3HBA breast carcinoma and sarcoma 37 grown in C3H and C mice respectively. The foregoing observations on sex hormone antagonisms at the mitochondrial enzyme level may offer some basis of mechanism for the numerous similar antagonisms noted at organ and body levels.

Paper chromatography of vitamin B_{12} and related bacterial growth factors. WALTER A. WINSTEN and EDWARD EIGEN (introduced by PHILIP B. HAWK). *Food Research Laboratories, Inc., Long Island City, N. Y.* In a preliminary note (letter to the *Journal of Biological Chemistry*), *Lactobacillus leichmannii* 313 has been reported to utilize six alternate growth factors. Two of these were found in vitamin B_{12} concentrates and presumably represent two forms of the vitamin. Hoffmann, *et al.* (*J. Biol. Chem.* 176: 1465, 1948) previously reported that crystalline APA factor (vitamin B_{12}) supports the growth of this organism. A third growth factor may be identified as thymidine, reported by Snell, *et al.* (*J. Biol. Chem.* 175: 474, 1948) to support the growth of *L. leichmannii* 313. The three other growth factors are of unknown composition. In the present report, the paper chromatographic procedure for separating the 6 alternate growth factors is described as well as the method of recognizing the positions of the several growth factors on a chromatogram by the use of *L. leichmannii* 313 as

a microbiological indicator. This technique allows one to interpret assay values for apparent vitamin B_{12} activity as measured in the tube microbiological assay procedure. Due to the very slow movement of the two forms of vitamin B_{12} on a paper chromatogram using n-butanol as the mobile phase, it is possible to separate these entities from the faster moving substitute growth factors. The isolated vitamin B_{12} entities may then be assayed by the usual tube assay procedure. Illustrative data are presented.

Metabolism of tissue cultures of mouse-brain in relation to propagation of Theiler's GD VII virus. RICHARD J. WINZLER and HAROLD E. PEARSON (by invitation). *Depts. of Biochemistry and Bacteriology, Univ. of Southern California School of Medicine and the Laboratory Division of the Los Angeles County Hospital, Los Angeles, Calif.* Sterile cultures of minced newborn mouse brain incubated at 35°C. are capable of supporting the multiplication of Theiler's GD VII strain of mouse encephalomyelitis virus. Studies on the oxygen consumption in Warburg vessels of such cultures with or without virus reveal no significant difference in the QO_2 values in the presence or absence of the virus over the period of most rapid virus growth (0–24 hours) and up to 72 hours. Similarly with cultures grown in Erlenmeyer flasks the presence or absence of virus had no influence on the rate of glucose utilization or of lactic acid production by the tissues. The rates of oxygen consumption, glucose utilization and lactic acid production fall rapidly with time and reach levels of about 10% of the original rates after 48 hours. In spite of this marked diminution of metabolism, the tissue retains its ability to support the growth of the virus for as long as 10 days of incubation before virus is added. It thus appears that the virus has little effect on the overall metabolism of the host tissue and that the rate of metabolism of the host tissue is not of paramount significance in governing the multiplication of the virus.

Acetoacetate production from hexanoic and other six carbon acids by rat liver preparations. ROBERT F. WITTER (by invitation) and ELMER STOTZ. *Dept. of Biochemistry, School of Medicine and Dentistry, The Univ. of Rochester, Rochester, N. Y.* Quantitative measurements of acetoacetate production from 6 carbon acids were made with rat liver homogenates and washed liver particulate matter. With the latter at *p*H 7.4–8.0, 1 mole of hexanoate yielded 1½ moles of acetoacetate, proving a recondensation mechanism. At *p*H 6–7 lower yields of acetoacetate were obtained at a slower rate, and a heat-stable factor from liver was necessary. The rate of acetoacetate formation from several possible intermediates of hexanoate oxidation was measured with both types of liver preparations at *p*H 8.0. Only α-hexanoate yielded acetoacetate at a rate comparable with hexanoate under the same conditions. In the washed liver system, sorbate, β-hydroxyhexanoate, β-hydroxy-γ-hexanoate, and β-ketohexanoate gave low rates of acetoacetate production. Upon addition of the heat-stable factor, these rates were all increased but only with sorbate did the rate approach that of hexanoate or α-hexenoate. The washed liver system, unlike the homogenate or the soluble extract (Witter and Stotz. *J. Biol. Chem.* 176: 501, 1948), did not metabolize triacetic (β,δ-diketohexanoic) acid. By use of a micro-bromination procedure for α-hexanoate, it has been found that its disappearance in liver homogenates is dependent on the presence of oxygen. Aerobically, β-hydroxyhexanoate did not inhibit the metabolism of α-hexanoate. The collected facts cast some doubt on a simple hydration of α-hexanoate and on β-hydroxyhexanoate as an intermediate. A study of various inhibitors is being made in an attempt to differentiate the steps in the production of acetoacetate from hexanoic acid.

Sulfur-free protein hydrolysates for cystine- and methionine-free diets. JOHN L. WOOD, SHIRLEY LUCILLE COOLEY (by invitation) and NELSON KINGSLAND (by invitation). *Dept. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis, Tenn.* In order to prepare a diet entirely devoid of organic sulfur, excepting for vitamins, a method for removal of all sulfur from protein hydrolysates has been devised. The protein hydrolysates were treated with freshly prepared Raney Nickel which removed all organic sulfur as nickel sulfide. Model experiments indicate that the cystine in the mixture was converted to alanine and the methionine to α-aminobutyric acid. A 15% solution of the hydrolysate was shaken with Raney Nickel for 3 hours at room temperature and *p*H 8.2. The solution was separated from the nickel and neutralized before concentration to dryness. The nickel adsorbed considerable of the nitrogenous products of the solution, but much of this was eluted by treatment with hydrogen sulfide. The ratio of amino nitrogen to total nitrogen in the final product was approximately the same as in the untreated hydrolysate. Qualitative tests for cystine and methionine and sulfhydryl were negative. Threonine analyses indicated that this amino acid was not destroyed by the alkaline treatment. A small amount of sulfate was found in the hydrolysate. In one instance this was removed with barium hydroxide to produce an entirely sulfur-free hydrolysate. Growth response in young rats demonstrated that essential amino acids other than methionine were retained in the desulfurized preparation. Both an acid hydrolysate and an enzyme hydrolysate have been desulfurized. When supplemented with methionine, or with cystine and methionine, each preparation supported

growth at approximately the same rate as the untreated hydrolysate.

Prolongation of pentobarbital sodium anesthesia by nitrates and nitrites. HAROLD WOOSTER (by invitation) and F. WILLIAM SUNDERMAN. *Mellon Institute, Pittsburgh, Penna., and Cleveland Clinic, Cleveland, Ohio.* The recovery time of white mice anesthetized with pentobarbital sodium is significantly prolonged by the intraperitoneal injection of nitroglycerine, butyl nitrate, diethyleneglycol dinitrate and sodium nitrite. Nitroglycerine appears to be the most effective of the compounds studied. The dosages required to demonstrate this phenomenon are of the order of 20–50 mg/kg. of body weight. Significant prolongation of recovery time may also be produced by the inhalation for 20 minutes of butyl nitrate in concentrations of 30–45 mg/l.

Interference of gentisic acid in determination of urinary uric acid after administration of salicylates. T. F. YÜ (by invitation) and ALEXANDER B. GUTMAN. *Research Service, First (Columbia Univ.) Division, Goldwater Memorial Hospital, and Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.* The chromogen removed by treatment of urine or serum with uricase is generally considered to be true uric acid. We find that in the urine of normal and gouty subjects receiving 5 gm. acetylsalicylic acid daily, as much as 25% of the total chromogens (as measured by urea-cyanide-carbonate and arsenophosphotungstic acid) may consist of a chromogen which is not uric acid but is destroyed by digestion with uricase, due to instability in the alkaline buffer. This chromogen was identified by countercurrent techniques as largely gentisic acid, showing essentially the same distribution between dilute HCl and a mixture of isopropyl ether and petroleum either as an authentic sample of gentisic acid. When 16 gm. of sodium gentisate was fed by mouth, the chromogenicity of the urine was tremendously increased; of this over 90% was gentisic acid. In determining uric acid in the urine of patients receiving salicylates, gentisic acid should first be removed. This can be accomplished by shaking an aliquot of acidified urine (*p*H 4.5–5.0) 2 or 3 times with 4 to 5 times the volume of ethyl ether. The gentisic acid is quantitatively removed in the ether extract.

Metabolism of isovaleric acid studied with isotopic carbon. IRVING ZABIN (by invitation) and KONRAD BLOCH. *Dept. of Biochemistry and Institute of Radiobiology and Biophysics, Univ. of Chicago, Chicago, Ill.* In continuation of earlier studies on the degradation of branched chain compounds and their possible role in sterol synthesis (*J. Biol. Chem.* 155: 255, 1944), isovaleric acid containing C^{13} in the two end methyl groups and C^{14} in the carboxyl group has been synthesized. After incubation of labeled isovaleric acid in rat liver slices ketone bodies were isolated according to Van Slyke and analyzed for C^{13} and C^{14}. The acetone fraction which includes acetone formed by decarboxylation of acetoacetate contained the C^{13} and C^{14} isotopes in a ratio of about 8 to 1. Two possible pathways for the formation of ketone bodies from isovaleric acid are *a*) loss of one of the end methyl groups with formation of a 4 carbon acid followed by oxidation of the latter to acetoacetate; *b*) oxidation of isovaleric acid at the tertiary carbon atom with production of acetone and a 2 carbon fragment. The 2 carbon fragment might condense to acetoacetate. The high absolute C^{13} concentration and the high C^{13}/C^{14} ratio found in the acetone fraction in the present experiment favors a pathway which involves splitting of isovaleric acid into three carbon and two carbon fragments. Cholesterol isolated from the same incubation experiment contained both carbon isotopes, the incorporation of C^{13} being significantly greater than that of C^{14}.

Distribution of radioisotopes of carbon and calcium administered to rats by continuous intraperitoneal infusion. S. H. ZBARSKY (by invitation), ROGER LIENKE (by invitation) and W. D. ARMSTRONG. *Dept. of Physiological Chemistry, Univ. of Minnesota Medical School, Minneapolis, Minn.* Studies were made of tissue uptake and excretion of C^{14} and Ca^{45} by rats receiving these isotopes by intraperitoneal infusion. The C^{14} was given as a solution of $Na_2C^{14}O_3$ in saline and the C^{14} in the expired air and excreta determined at intervals over a period of 5 days. The C^{14} content of the expired air rapidly reached a high value which was maintained throughout the injection. Over 90% of the injected C^{14} was excreted in the expired air and the C^{14} contained in the urine was present chiefly as urea. The specific activities of the tissues and tissue components were found to be higher than in earlier experiments in which rats received intermittent injections of $Na_2C^{14}O_3$ or intraperitoneal implants of $CaC^{14}O_3$. The Ca^{45} was given over a period of 3 days as a solution of $Ca^{45}Cl_2$ in saline. Feces and urine were collected daily during this time and for 5 days after the infusion was stopped; Ca^{45} appeared in all samples of feces and the total fecal excretion of Ca^{45} was nearly equal to the amount in the urine. The Ca^{45} present in the skeleton, teeth and other tissues was determined. The results and conclusions derived from these and other similar experiments will be presented.

Effect of antimalarial agents on adenosine triphosphatase of snake venoms. E. ALBERT ZELLER (introduced by MARSCHELLE H. POWER). *Division of Biochemistry, Mayo Foundation, Rochester, Minn.* Many snake venoms are able to catalyze the hydrolysis of one phosphoric acid from ATP.

This reaction is activated by some bivalent ions like magnesium, calcium, manganese and cobalt, and is inactivated by some other ions such as zinc, copper and mercury (E. A. Zeller, 1948). Hydrogen sulfide, cysteine and ascorbic acid inhibit the reaction while sodium cyanide activates it, possibly by removing inhibiting heavy metals. Histamine inactivates the enzyme considerably in 0.001M solutions. Other diamines, like pyridoxamine, have the same effect (E. A. Zeller. *Proc. Soc. Exper. Biol. & Med.*, 1949). It has been observed recently that still other drugs which are derivatives of diamines also influence ATPase in a similar way. Thus, stilbamidine in 0.004M solution produces 63% inhibition of ATPase from *Bitis gabonica*, regardless of the degree of activation of the enzyme by magnesium ions. An approximately similar degree of inhibition of this enzyme is also produced by 0.003M quinine and 0.0015M atabrine in the presence of various concentrations of magnesium ion. It is of interest to note that dephosphorylation of ATP by homogenates of guinea-pig adrenal glands was likewise inhibited to a similar extent by stilbamidine and atabrine.

Non-choline esters as substrates of e-cholinesterase ('true' cholinesterase). E. Albert Zeller, Gerard A. Fleisher and Robert A. McNaughton (introduced by Marschelle H. Power). *Division of Biochemistry, Mayo Foundation, Rochester, Minn.* It has been shown that cholinesterase, which is present in certain snake venoms, is of the 'e' type (Zeller. *Helvet. chim. acta* 33: 1949) and that it is able to hydrolyze ethyl haloacetates (Zeller and Utz, *Helvet. chim. acta* 33: 1949). The same result has been obtained with ethyl chloro-acetate and e-cholinesterase of human erythrocytes (McNaughton and Zeller. *Proc. Soc. Exper. Biol. & Med.*, 1949). The use of the abovementioned esters has two disadvantages: *1*) the enzymatic reaction is rather slow as compared to that obtained with acetylcholine and *2*) the substrate blanks are relatively high. We looked, therefore, for better non-choline substrates of cholinesterase. β-Chlorethyl acetate was hydrolyzed by different snake venoms (*Naia naia, Naia melanoleuca* and *Pseudechis prophyriacus*), and erythrocyte cholinesterase at a suitable rate. The non-enzymatic blanks were much lower than those of the other substrates. Enzymatic hydrolysis of this compound and of acetylcholine was inhibited to the same degree by physostigmine. Simultaneous hydrolysis with different concentrations of this non-choline ester and acetylcholine always resulted in competitive inhibition, both with hemolyzed erythrocytes and with snake venom. Purified cholinesterase from human erythrocytes (which, contrary to the unpurified hemolyzed erythrocytes, does not split methyl butyrate) also catalyzed the hydrolysis of β-chloroethyl acetate, and gave rise to competition with acetylcholine. The β-Chloroethyl acetate was split more quickly by e-cholinesterases (snake venoms, erythrocytes) than by s-cholinesterases ('pseudo-' cholinesterase) from human serum or guinea-pig parotid glands.

Effect of ultrasonic vibrations on the release and inactivation of paramecin. L. P. Zill (by invitation) and W. J. van Wagtendonk. *Dept. of Zoology, Indiana Univ., Bloomington, Ind.* Experiments were carried out to determine the feasibility of using high frequency ultrasonic vibrations as a method of disintegrating large numbers of Paramecium aurelia as a preliminary step in the isolation of paramecin ('killer' substance of *Paramecium aurelia* 51, variety 4). The instrument used had a maximum input of 1000 watts at a frequency of 450,000 cycles/sec. Tests which were run on whole animals demonstrated that complete disintegration was not obtained with maximum input for five minutes. Paramecin was completely inactivated during this treatment. In order to investigate ultrasonic effects on paramecin itself, experiments were performed, using a brei prepared by repeatedly forcing a suspension of Paramecia through a narrow-gauge injection needle. It was found that ultrasonic treatment at low intensity increased the activity of the original brei. The maximum increase in activity was found to be obtained with a constant current of 80 milliamperes (ca. 32 watts) and at a bath temperature not exceeding 6°C. All further experiments were performed under identical conditions of vibration (constant current of 80 milliamperes). If vibration was carried out under an atmosphere of air or oxygen, increases in activity up to 185% were observed. No increase or decrease in the apparent activity of the brei was found if the vibration took place under an atmosphere of nitrogen. When oxygen was passed through the sample during vibration, the inactivation of paramecin proceeded according to a first order reaction.

Polarographic micro-method for determination of chloride in whole blood, serum and plasma. Walter J. Zimmerman and William M. Layton, Jr. (introduced by H. Jensen). *Medical Department, Field Research Laboratory, Fort Knox, Ky.* A polarographic method has been developed for the determination of chloride concentration in from 0.02 to 0.05 ml. of whole blood, plasma and serum. Proteins are removed by an adaptation of the Somogyi barium hydroxide-zinc sulfate precipitation, the final dilution of the sample for analysis being 1:100 in 0.1N potassium nitrate. The diffusion current, determined by a manual polarograph at an applied voltage of 0.0 volts against a saturated mercurous sulfate half cell has been found to be directly proportional to chloride concentration in the range of concentration used. It is unnecessary to calibrate the apparatus in absolute

units or to thermostat the analysis cell, provided the diffusion current is determined for two standard chloride solutions. The method has been tested for accuracy by means of duplicate determinations on 0.05 ml. of plasma with a mean error of 0.6% and by addition of 40 millimoles of potassium chloride with a mean error in recovery of 0.8%. Plasma chloride values obtained by the polarographic method have been found to correspond with values obtained with identical solutions by a standard volumetric technic. The polarographic method has the advantage of utilizing small volumes of blood, thus allowing frequent serial chloride determinations on small laboratory animals without causing undesirable changes attending excessive blood loss.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INC.

Thirty-ninth Annual Meeting

DETROIT, MICHIGAN, April 18–22, 1949

(For possible corrections in any of the following abstracts see the June issue)

In vitro oxidation of morphine by tissue homogenates. L. G. ABOOD and ERNEST KUN (introduced by JULIUS M. COON). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* Isotonic tissue homogenates prepared in Krebs-Henseleit solution oxidize morphine *in vitro*. Morphine derivatives in which the phenolic hydroxy group is substituted are not oxidized, but depress the endogenous oxygen uptake. The oxidation of morphine sulfate was followed manometrically and colorimetrically by determining the reducing value of the flask contents. In 1 hour 70 to 80% of the added morphine was oxidized, a fact which corresponds to an equivalent decrease in the reducing value of the reaction mixture (10–100 mg. of tissue homogenate plus 10 mg. of morphine sulfate), suggesting that the phenolic hydroxy group is oxidized probably to a quinone. On this basis 1 mole of morphine utilizes one-half atom of oxygen, which is in agreement with the experimental findings. The oxidized morphine is still pharmacologically active, and it is not further oxidized by tissue preparations. Muscle, brain, liver, kidney of rabbit, rat, and guinea pig were all able to oxidize morphine. Invertebrate tissues also oxidize morphine. The extra oxygen consumption of 10 mg. of morphine is depressed 59% by 0.01M NaCN, 83% by 0.01M Na_2SeO_3, while no inhibition occurred with 0.01M NaN_3 or 0.001M $HgCl_2$.

Effect of morphine sulfate on cerebral blood flow and metabolism in man. BENEDICT E. ABREU, HENRY W. ELLIOTT, VIOLETTE C. SUTHERLAND (by invitation), LESTER MARGOLIS (by invitation) and ALEXANDER SIMON (by invitation). *Divisions of Pharmacology and Experimental Therapeutics and of Psychiatry, Univ. of California Medical School, San Francisco, and Langley Porter Clinic, State Dept. of Mental Hygiene, San Francisco, Calif.* Mean arterial blood pressure (MBP), cerebral blood flow (CBF), cerebral O_2 and glucose uptake and CO_2 output were determined in 5 subjects before and 20 minutes after morphine SO_4, 10 mg., i.m. All were voluntary patients who were in the Langley Porter Clinic for psychiatric care. Results expressed as percentage change from the control values are indicated in the table.

Pt.	Sex	M.B.P.	C.B.F.	O_2	CO_2	Glucose
M. R.	f	−7	−21	−32	−19	0
M. P.	f	+9	−22	−31	−25	−19
R. R.	m	+3	−3	+4	−2	+124
S. M.	m	0	−9	−17	−26	+40
J. T.	m	+3	+58	+60	+47	+124

The trend of effect as indicated in 5 subjects is for morphine to decrease significantly the CBF (in 2 of 5), O_2 uptake (in 3 of 5), CO_2 output (in 3 of 5), but to increase glucose uptake (in 3 of 5).

Simple method for determining the degree of vascular sympatholytic action. RAYMOND P. AHLQUIST. *Dept. of Pharmacology, Univ. of Georgia School of Medicine, Augusta, Ga.* The increasing use of sympatholytic agents as tools in pharmacological and physiological research has demonstrated the need for a simple method for determining more exactly the degree or extent of sympatholysis present in the experimental animal. The proposed method is based on the reduction, by sympatholysis, of the intense vasoconstriction produced by the injection of epinephrine, 0.005 mg/kg., into the femoral artery. This dose, which practically always produces a marked pressor response when injected intravenously, has little or no pressor effect when injected into the femoral artery of untreated dogs. As sympatholysis develops following the administration of Dibenamine, Priscol or other sympatholytics, this intraarterial constricting effect becomes diminished; and the pressure response to the intra-arterial injection becomes more similar to the intravenous response. This method gives much more information regarding degree of sympatholysis than does a determination of the presence or absence of epinephrine 'reversal'. This is well illustrated by the finding that even though epinephrine 'reversal' is present, the intra-arterial injection shows that epinephrine is still capable of producing marked vasoconstriction. It has also been found that in some of the visceral vascular beds the vasoconstricting action of epinephrine cannot be prevented by any dose of the sympatholytic agents tested thus far.

Sympatholytic action of dibenamine. RAYMOND P. AHLQUIST. *Dept. of Pharmacology, Univ. of Georgia School of Medicine, Augusta, Ga.* Nickerson

et al. first showed that epinephrine, even in very high dosage, produced only a depressor response when injected intravenously into cats or dogs following an effective dose of Dibenamine. Although this tends to show that the sympatholytic action of Dibenamine is absolute against epinephrine constriction, results obtained in this laboratory, using direct blood flow measurements and intra-arterial injections of epinephrine, show that the action of Dibenamine is not absolute. Epinephrine continued to produce constriction in the vascular bed of the pregnant dog's uterus following doses of Dibenamine up to 40 mg/kg. In these same experiments the epinephrine also produced uterine contractions after Dibenamine. In some dogs it was found that the femoral intra-arterial injection of epinephrine continued to produce vasoconstriction following high doses of Dibenamine although the usual response in this case is a slight vasodilation. Other vascular beds are still under investigation. These results emphasize the fact that the response to epinephrine following sympatholytic agents is determined by the ratio between the receptors which respond unchanged to epinephrine (vasodilation, uterine inhibition etc.) and the receptors whose response is decreased but apparently not completely abolished (vasoconstriction, uterine stimulation, etc.) These results also indicate that the apparent differences between so-called 'adrenolytic' and 'sympatholytic' action are probably not real since intra-arterial injection of epinephrine corresponds more closely to sympathetic nerve stimulation (other than to the adrenal medulla) than does intravenous injection.

Influence of orally administered rutin on adrenal ascorbic acid storage in guinea pigs. ANTHONY M. AMBROSE and FLOYD DEEDS. *Pharmacology Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, USDA, Albany, Calif.* In a previous paper we have shown that guinea pigs maintained on a scorbutogenic diet and receiving suboptimal doses of ascorbic acid (0.2 mg/day) in conjunction with rutin (100 mg/day) survived longer and showed less severe scorbutic symptoms than guinea pigs receiving ascorbic acid or rutin alone. In the light of results by Cotereau *et al.* (*Nature* 161 : 557, 1948) on the 'sparing action' of vitamin P or catechin on adrenal ascorbic acid, it was felt that this approach to the evaluation of the physiological role of rutin might be more objective. Accordingly, 120 young guinea pigs were divided into 4 groups of 30 each and placed on a scorbutogenic diet. In addition the following supplements were administered orally each day. One group served as control, one group received 100 mg. rutin in 0.25 ml. propylene glycol, one group received 2 mg. ascorbic acid, and another group received 2 mg. ascorbic acid and 100 mg. rutin. After 12 to 20 days, 8 animals from each group were sacrificed and adrenal ascorbic acid determined by the method of Roe and Kuether. Our results indicate that rutin appears to play no important role as an 'economizing factor' in the storage of adrenal ascorbic acid under the conditions of our experiments. Smaller doses of ascorbic acid, 1 mg. or 0.5 mg/day, clearly demonstrate that as the dose of ascorbic acid is lowered there is an accompanying lowering of the amount of adrenal ascorbic acid stored irrespective of rutin.

Aureomycin: protective effect in experimental Rocky Mountain spotted fever with particular reference to intermittent treatment. LUDWIK ANIGSTEIN, DOROTHY M. WHITNEY, and JOE BENINSON (introduced by G. A. EMERSON). *Rickettsial Research Laboratory, Dept. of Preventive Medicine and Public Health, Univ. of Texas Medical Branch, Galveston, Texas.* Time-dose relationships in intermittent aureomycin therapy of spotted fever is the subject of this study. A total of 30 guinea pigs were infected with spotted fever and subsequently injected subcutaneously with repeated single doses of 1.0, 2.0, and 3.0 mg. of aureomycin hydrochloride (Lederle) per guinea pig (weighing 450–500 gm.), at 48- and 72-hour intervals. Four injections were given to each test animal covering the incubation and most of the febrile period of the controls. A drastic change of the clinical pattern of the infection was manifested by all treated guinea pigs. The fever was either entirely suppressed or reduced to abortive attacks after long incubation, regardless of time-dose schedule. Whereas the untreated controls developed typical spotted fever and died, all test animals recovered with immunity to reinoculation with spotted fever. Evidence was thus gained that intermittent aureomycin therapy with minimum dosage is equally efficient in spotted fever as the continuous massive treatment. The protective effect of aureomycin and the ultimate immunity of the infected guinea pig express the balance kept by the antibiotic between the rickettsial invader and the host resistance. This was substantiated by the specific antibody response in the mechanism of the rickettsiostatic activity of the antibiotic, as found by complement fixation tests. This phase of the problem is under study.

Physiological disposition of aminopyrine in man. JULIUS AXELROD (by invitation) and BERNARD B. BRODIE. *Dept. of Biochemistry, New York Univ. College of Medicine, New York Univ. Research Service, Goldwater Memorial Hospital, and Laboratory of Industrial Hygiene, New York City.* Aminopyrine is quickly and essentially completely absorbed from the gastro-intestinal tract. Only about 2% of the drug is excreted unchanged indicating its almost complete metabolism in the body. Plasma levels decline slowly, about 15 to 20% per hr. The route of metabolism of amino-

pyrine was shown to be as follows: About 55% of the drug demethylates to form 4-aminopyrine, which in turn is acetylated and excreted in this form. The other route or routes of metabolism were not ascertained. Preliminary results suggest that 4-aminoantipyrine is an effective antipyretic, antirheumatic and analgesic.

Differentiation of sympathetic from parasympathetic drugs by measurements of phaseboundary potentials. T. C. BARNES and R. BEUTNER. *Hahnemann Medical College and Hospital of Philadelphia, Philadelphia, Pa.* The oil-cell method (Barnes and Beutner, *Science* 104: 569, 1946) provides a basis for the distinction between adrenergic and cholinergic nerves. Previous reports (*Federation Proc.* 4: 112, 1945, *Internat. Physiol. Congress* 1947, p. 60) showed that adrenergic drugs dissolve in triglyceride oils on which they form negative phaseboundary potentials. In contrast, the cholinergic drugs choline, acetylcholine, mecholyl, pilocarpine, eserine, prostigmine, etc. are inactive on adrenergic oils. Tests show that atropine, curare and etamon also have the solubility of cholinergic drugs. The alipathic sympathomimetics give potentials with the adrenergic oils. (In this abstract all concentrations are 0.05% and potentials negative). Aranthol gave 19 mv. on tributyrin, octin produced 32 mv. on triacetin and oenethyl gave 15 mv. on triacetin. Adrenergic blockade may be competitive negativity since priscol produced 45 mv. on tributyrin, yohimbine 77 mv. on tributyrin and dibenamine 6 mv. on triacetin. Most ergot compounds are difficult to test in the oil-cell but ergonovine gave 53 mv. on the adrenergic oil tributyrin. It is interesting that nicotine has the solubility characteristics of adrenergic drugs giving 87 mv. on tributyrin suggesting that the pressor effect is direct adrenergic action. The problem of nicotinic and muscarinic effects is now being investigated by the oil-cell method.

Relation of 4-methyl and 4-ethyl substituted imidazoles to: *1*) histaminic action; 2) antagonism by antihistaminics. W. BARRETT (by invitation), J. FEICKERT (by invitation), B. CRAVER. *Division of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.* In respect to the relation between chemical structure and histaminic activity as judged by the stimulating action of these compounds upon the smooth muscle of the ileum of the guinea pig, the following observations seem justifiable: the activity appeared progressively to diminish as one or both of the hydrogens of the amino group were replaced by alkyl groups, and the length of these alkyl groups was extended; the replacement of the amino group with other groups diminished or eliminated the activity; the amino methyl derivative of imidazole was devoid of this action unless at least one hydrogen were substituted by an alkyl group, but otherwise the effect of increasing the length of the alkyl group was the same as in the ethyl series. As to the relation between the chemical structure of these compounds and the antihistaminic activity of Pyribenzamine, the following conclusions appear justifiable: minimal doses eliminated the histaminic action of the compound in the ethyl series only in the presence of an amino group, whether it were mono- or di-substituted, but in the methyl series the low doses of Pyribenzamine sufficed only if but one hydrogen had been replaced by an alkyl group and not if both had been replaced. Its antagonism in high doses toward other derivatives would appear attributable to its mild anticholinergic activity.

Effect of certain mitotic poisons on uric acid and allantoin excretion in mice. ALLAN D. BASS and ELIZABETH F. PLACE. *Dept. of Pharmacology, College of Medicine, Syracuse, N. Y.* Kopac (*Tr. N. Y. Acad Sci.* 8: 5, 1945) has called attention to the relationship of mitotic poisons to nucleoprotein systems. Krizik *et al.* (*U. S. Atomic Energy Commission Report* MDDC 1647) found no increase in uric acid or allantoin in the urine of dogs given lethal doses of X-ray, a well established mitotic inhibitor. These observations appeared to be somewhat at variance in light of the reduction in amount of lymphoid tissue we have observed in mice following administration of such mitotic poisons as nitrogen mustard and urethane. A study was therefore begun to determine in mice the effect of mitotic poisons on the products of nucleoprotein catabolism. C3H or $C3H_{F1}$ hybrid mice bearing 6C3HED tumors were used since these lymphoid tumors gave a large reservoir of lymphoid tissue high in nucleoproteins. Urine was collected from groups of 10 to 15 animals over a period of three hours. Daily doses of colchicine (0.75 mg/kg.) and nitrogen mustard (2 or 4 mg/kg.) in saline were administered intraperitoneally. Uric acid-creatine and allantoin-creatinine ratios were determined since only fractions of the 24-hr. output of urine were collected. The uric acid-creatine ratio was elevated in the 6 to 48-hour period following drug administration. The allantoin-creatinine ratio was elevated to a lesser extent. Less striking but definite changes were observed in non-tumor bearing animals. Blood uric acid levels were also found to be elevated following administration of the agents used. The findings suggest that no major defect in nucleoprotein catabolism is affected by the mitotic poisons studied.

The subcutaneous use of thiomerin, a new mercurial diuretic for treatment of congestive heart failure. ROBERT C. BATTERMAN, DAVID UNTERMAN (by invitation) and ARTHUR C. DEGRAFF. *Dept. of Therapeutics, New York Univ. College of Medicine, New York City.* The diuretic effectiveness in terms of predictability of

response, and degree of diuresis was ascertained for a new mercurial diuretic, Thiomerin, N(β-carboxymethyl mercapto - mercuri - γ - methoxy) propyl camphoramic acid disodium salt. In a group of 45 patients with congestive heart failure, the subcutaneous administration of Thiomerin resulted in a satisfactory diuresis in 86.6% of trials as compared with 81.0 and 83.3% of the trials for Thiomerin and Mercuzanthin respectively, given intravenously. The degree of diuresis for each drug regardless of route of administration was equivalent, indicating that complete absorption occurred from the site of subcutaneous injection. In an additional group of 35 ambulatory and 73 bedridden patients, Thiomerin when administered subcutaneously was satisfactory in removing the edema, preventing the accumulation of edema and for maintenance of patients' state of compensation. In all 589 injections of 1 to 4 cc. of Thiomerin were given subcutaneously to 143 hospital and ambulatory patients. The drug was well tolerated by the patients in 90% of the trials. Subjective burning at the site of injection occurred in approximately 10% of the number of injections. Tender induration and nodular formation was noted in 4 and 2.5% respectively. Thiomerin when given subcutaneously is an effective and safe diuretic with a predictability of diuretic response and degree of diuresis equivalent to that noted with other mercury diuretics used intravenously and is superior to these preparations used intramuscularly.

Subacute respiratory toxicity of a new insecticide activator in 'aerosol bomb' mixture. ROBERT O. BAUER and WALTER W. JETTER (introduced by GEORGE L. MAISON). *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.* Animals were exposed in a closed chamber to dispersions of insecticide mixtures in air 1 hour a day for 4 weeks. The mixture contained (activator) N-octyl bicycloheptene dicarboximide 2%, (insecticide) DDT 2% in kerosene) 1%, (vehicle) methylene chloride 5%, alkyl naphthalenes 5%, freons 11 and 12, 85%. Two concentrations at 1 gm. and 0.1 gm/60 l. air were used. Ten guinea pigs and 10 Wistar rats were subjected to each concentration. An equal number served as controls. Hemoglobin, red and white cell counts, urinary albumin and sugar were estimated before and after the experiment. The test animals displayed irregular food consumption. Group growth curves were depressed. Surviving test animals regained lost weight completely. Only 4 fatalities occurred and these were in rats on high concentrations. In both species fine tremors, hyperirritability and conjunctival hyperemia were observed, becoming progressively worse and disappearing after exposure. Female guinea pigs receiving high concentrations developed reversible alopecia. Mild anemia in test guinea pigs and leucopenia in test rats were encountered irregularly. Incomplete pathologic examinations show: *1*) moderate to severe interstitial pneumonitis in all test animas; *2*) focal necrosis of liver and spleen in high concentration rats; *3*) extensive splenic hemosiderosis and hypoplastic bone marrow, chiefly in high concentration guinea pig group. Pneumonitis and conjunctival hyperemia in test animals point to direct chemical irritation, more likely due to the vehicle than the insecticide or activator. Other clinical and pathologic changes may result from the insecticide mixture, nutritional disturbance or a combination.

Survival of plasmodium cathemerium sporozoites in hen blood with addition of canary blood cells. HARRY BECKMAN. *Marquette University School of Medicine, Milwaukee, Wis.* Mosquitoes carrying the sporozoites of *P. cathemerium 3H2* were ground in Locke's solution and added simultaneously to freshly prepared hen plasma to which there had been added to respective portions *a*) freshly washed canary blood cells, *b*) freshly washed hen blood cells, *c*) a mixture of freshly washed canary and hen blood cells. The three mixtures were incubated with gentle agitation at 41.5° C. for 1 hour and then portions representing 1 mosquito each were withdrawn and injected intramuscularly into canaries, the peripheral blood of these birds being subsequently examined from the 5th to 16th days, inclusive. Birds failing to become positive were challenged by mosquito bite. Trials employing 4 birds in each group were run at sufficient intervals that at least one complete 3-week sporozoite-to-sporozoite cycle lay between. Of the 16 canaries injected with hen plasma plus canary cells, all developed patent blood infections. Of the 16 injected with hen plasma plus hen cells, none developed patent infections and all became infected when later challenged by mosquito bite. Of the 16 birds injected with hen plasma plus both hen and canary cells, 13 developed patent blood infections. It would appear from this preliminary study that it is the formed elements of canary blood that permit survival of sporozoites in hen plasma, in which alone they do not survive, as earlier shown in this laboratory.

Some metabolic changes observed in seizures induced by certain agenetreated protein in dogs. JULIUS BELFORD (by invitation) and D. D. BONNYCASTLE. *Laboratory of Pharmacology, Yale Univ., New Haven, Conn.* The resemblance to human epilepsy of the motor seizure and E.E.G. dysrythmia occurring in canine 'epilepsy' (caused by certain 'agene' (NCl_3)-treated proteins such as wheat gluten and zein, etc.), warranted further study of this seizure phenomena. Five to 8 dogs were employed in each experiment and were classified as being in the normal, pre-fit, fit, (and when feasible) inter-paroxysmal stage. Blood samples

were taken from these animals and examined for various entities. These results were subjected to statistical examination. No significant changes were found at these several stages in serum calcium, magnesium or choline esterase. The intravenous injection of 100 mg. of physostigmine, reputed to lower serum choline esterase by 95% did not precipitate convulsions; on the other hand, sub-convulsive doses of metrazol could induce a seizure. No significant differences were found in arterial glucose, but a significant pre-fit fall occurred in venous glucose followed by a rise during a seizure. No significant A-V glucose differences were observed although there was a tendency towards negative A-V differences in the abnormal phases. The oxygen content of arterial and venous blood showed no change. However, while the arterial carbon dioxide did not change, the venous carbon dioxide fell significantly at the time of the fit.

Observations on administration of BAL in selenium poisoning. J. B. BELOGORSKY (by invitation) and DONALD SLAUGHTER. *Dept. of Physiology and Pharmacology, Univ. of South Dakota School of Medicine, Vermillion, S. D.* Braun, Lushy and Calvery, (*J. Pharmacol. & Exper. Therap.* 87: 119, 1946) reported that BAL was not only ineffective in the treatment of selenium poisoning but actually increased the toxicity of this element in experimental animals. Because of the prevalence of selenium in the soil of this region, further work seemed pertinent. Twenty young rats were used in each of three experiments; sodium selenite (SS) was administered intraperitoneally, BAL intramuscularly. Animals were injected daily for a period of 14 to 24 days. Varying doses of SS caused no toxic symptoms until a level of 10.0 mg/kg. was reached. When a non-toxic dose of BAL (15.0 mg/kg.) was administered ½ hour following the injection of a nontoxic dose of SS, (5.0 mg/kg.) all animals died. This would seem to indicate that BAL and SS have a synergistic toxicity. Durlacher *et al.*, (*J. Pharmacol. & Exper. Therap.* 87: 28, 1946) state that BAL is toxic in a 15 mg/kg. dose but our work does not bear out this statement. As a matter of fact, histological examination of the liver following BAL alone reveals no pathological changes. Since the liver is considered to be the probable site for detoxification of SS, it is of unusual interest to observe that BAL prevented the necrosis and fat deposits produced by SS alone. Further experimental work to elucidate this seeming paradox is being conducted.

Alteration of the activity of the human gastrointestinal tract with dihydroergocornine methanesulfonate (D.H.O. 180). W. D. BENNETT (by invitation), L. E. MORRIS (by invitation) and O. SIDNEY ORTH. *Depts. of Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* Administration of the dihydroergot derivatives, particularlydihy droergocornine methanesulfonate (D.H.O. 180—Sandoz), may elicit initially unfavorable side effects such as anorexia, nausea and vomiting. When this drug was given to block possible cardiac irregularities before cyclopropane anesthesia, it was noted subsequently in patients in which laparotomies were performed that the intestines were quite contracted; in fact that they had a ribbon-like appearance. It seemed possible that such evident stimulation of the gastrointestinal tract could be used to advantage clinically if the proper amount of one of these relatively non-toxic dihydroergot compounds was used. Dihydoergocornine has been administered in doses of up to 4 mg. total intravenously given in about 4 minutes; 1–2 mg. as a single dose intramuscularly; or 3 mg. q.i.d. orally. In cases of intestinal distention, intestinal obstruction, and congenital megacolon, this drug has been found to be distinctly advantageous.

Paralysing action of 2-methyl, 2-n-amyl-4-hydroxymethyl-1,3-dioxolane (glyketal). F. M. BERGER (introduced by ARNOLD D. WELCH). *Univ. of Rochester School of Medicine, Rochester, N. Y.* Numerous 2-substituted-4-hydroxymethyl-1,3-dioxolanes possess central depressant properties similar to those of the glycerol ethers or benzimidazoles. 2-methyl,2-n-amyl-4-hydroxymethyl-1,3-dioxolane appeared most active in this respect. This compound in doses of 100–150 mg/kg. injected intraperitoneally to small laboratory animals caused paralysis of the voluntary muscles. During paralysis respiration was not depressed and the animals remained conscious. There was a marked decrease of muscular tone. The muscles remained excitable to direct and indirect stimulation and there was no curare-like action. The corneal and pupillary reflexes and the knee jerk remained unaffected. The animals did not react to painful stimuli of moderate intensity. This was probably due to the motor paralysis. The compound had no analgesic action in nonparalytic doses. Paralysis was followed by complete recovery of all functions. After lethal doses death was due to respiratory paralysis. On intravenous injection mild and transient peripheral vasodilatation was caused. The action of acetylcholine was not interferred with. Glyketal also had a local anesthetic and quinidine-like action. Its anticonvulsant action against strynchnine was of a similar order as that of myanesin. Myanesin and glyketal differed in their metrazol antagonism. Myanesin had little effect on the occurrence of convulsions but protected the animals from death. Glyketal behaved as a true, although inefficient, anticonvulsant by protecting the animals from both convulsions and death.

Ratio of amino acid nitrogen to creatinine in the urine as a sensitive diagnostic test for ura-

nium poisoning in the rabbit. H. BERKE AND A. ROTHSTEIN (introduced by H. C. HODGE). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* It has been shown in this laboratory that the ratio of amino acid nitrogen to creatinine in the urine was a sensitive indicator of uranium poisoning in the rabbit. During daily exposure to inhalation of uranium dusts, the ratio remained elevated in contrast to the more or less rapid decline of other blood and urine constituents which also became abnormally high soon after exposure to uranium. More detailed experiments, therefore, were set up to determine the animal response in terms of this ratio to intravenous administration of uranyl nitrate. The minimal dose of uranyl nitrate which gave a statistically significant increase in ratio was 0.02 mg. U/kg. The ratio was a more sensitive test than either urinary protein or blood NPN. Blood amino acid nitrogen remained normal in all animals, indicating that the amino aciduria was renal in origin. Multiple injections of UO_2NO_3 at 2- to 3-week intervals at levels of 0.04, 0.1 and 0.2 mg. U/kg showed 3 patterns of response as measured by the ratio. In general the magnitude of the response was proportional to the dose given. At lower levels of administration, the maximal increase in the ratio occurred soon after each injection of uranium followed by lower but still high values. The peak values following each injection either remained the same, declined or increased in value depending on the dose given. A few animals, observed for 3 months after their last multiple injection, still showed abnormally high values of the ratio, even though histological examination revealed no renal damage directly attributable to uranium.

Effect of a number of aralkylamines on tyramine oxidation by amine oxidase. FREDERICK BERNHEIM and EDWIN J. FELLOWS. *Duke Univ. School of Medicine, Durham, N. C. and Temple Univ. School of Medicine, Philadelphia, Pa.* The present experiments were carried out in order to determine if a correlation existed between the central excitatory and amine oxidase inhibitory activity of a number of benzylamine, beta-arylethylamine and aryl-2-aminopropane modifications. In many instances an excellent correlation was observed, however, there were notable exceptions. Injection of several 3-amino-1-phenylbutane and 4-amino-1-phenylpentane derivatives and subsequent histopathological examination of the brain and cord of these animals disclosed a destructive effect on certain cells of the nervous system. Since enzymatic data on this type compound were not available, it appeared desirable to include them in the present studies. All of these agents exhibited a significant amine oxidase inhibitory effect.

Chemotherapeutic effect of 8-amino quinolines in schistosoma mansoni infections in mice. RAYMOND N. BIETER, ELIZABETH M. CRANSTON, WAYNE CHADBOURN (by invitation), ASHTON C. CUCKLER (by invitation), DOMINIC DEGUISTI (by invitation), W. W. BECKLUND (by invitation), and HAROLD N. WRIGHT. *Dept. of Pharmacology, Univ. of Minnesota Medical School, Minneapolis, Minn.* Two 8-amino quinolines, 6-methoxy-8-B diisobutylaminoethylamino quinoline dihydrochloride (SN2842) and 6-methoxyl-8-diisobutylaminoisopropylamino quinoline dihydrochloride (SN-3501), have been found to possess a relatively high degree of chemotherapeutic activity in schistosomiasis in mice. The drugs were administered in the diet in varying concentrations to mice injected intraperitoneally with 100 to 125 cercariae of schistosoma mansoni shed from snails of the species Australorbis glabratus. Therapy was started 4 to 6 weeks after infection and continued for 1, 2, or 4 weeks. Mice were autopsied 1 or 2 weeks after therapy was discontinued. The number of worms in the portal and mesenteric veins and liver were counted. When mice were treated with SN3501 in a concentration of 0.1% for 2 weeks 85% of 32 mice contained no worms at autopsy, compared with 10% negativity in the controls, and the average number of worms per mouse was 0.25, compared with 8.4 per mouse in the controls. With SN2842 in the same concentration and for the same duration of therapy 97% of 39 mice were completely negative for worms, compared with 12% of control mice, and the average number of worms per mouse was 0.1, compared with 9.5 per mouse in the controls. Eleven other 8-amino quinolines tested were found to be relatively inactive. Neither SN3501 nor SN2842 showed activity as a prophylactic when administered simultaneously with infection for a period of 2 weeks in a concentration of 0.1%.

Depressor action of nitrate esters of alkyl glycollates. JOSEPH G. BIRD (by invitation), HARRY K. IWAMOTO, JOHN B. HARMON (by invitation) and JOHN C. KRANTZ, JR. *Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore, Md.* A series of nitrate esters of glycolic acid esterified with alkyl alcohols varying from methyl to decyl were prepared. Certain physicochemical properties associated with pharmacodynamic responses were investigated. Depressor responses elicited, in dogs under anesthesia, by these esters were observed. It appears that the depressor response in the series studied increased with decreasing water solubility of the higher esters.

Quality and potency determination of analgetics. D. D. BONNYCASTLE and JOHANNES IPSEN, JR. (by invitation). *Laboratory of Pharmacology, Yale Univ., New Haven, Conn.* Experiments are reported in which a constant heat stimulus was

applied to the rat tail and the log-ratio of reaction time before and during the influence of a drug was used as a metameter of response to pain. The mean response of groups of such animals was calculated from truncated distributions. The relation of reaction time to degree of stimulus also was studied. The curves illustrating response/log dose relationship for the individual drugs take various forms, and three main types can be recognized. In one group, (e.g. acetylsalicylic acid) the continuous rise in response levels off asymptotically at about 30% increase in reaction time. A second type (e.g. codeine) presents the same picture at lower doses; but with high doses a secondary rise occurs tending towards an asymptote at about 100%. A third form of reaction curve is a direct rise from 0 to an asymptote of 100%. Intermediate forms between these types may be observed. It is suggested that the two asymptotic levels represent respectively the maximum analgetic, (thalamic?) effect and the maximum hypesthetic (cortical?) effect. These two properties of the drug are measured by the two doses at the respective points of inflection in the first and secondary rises. In the description of the pain-relieving activity of an individual chemical compound these two constants may serve to indicate *a*) simple analgetic potency, and *b*) narcotic activity, respectively.

Binding of streptomycin to plasma proteins. G. E. Boxer, V. C. Jelinek (by invitation), and A. O. Edison (by invitation). *Research Laboratories of Merck & Co., Inc., and the Merck Institute for Therapeutic Research, Rahway, N. J.* Elsewhere it has been reported that the clearance of streptomycin is about 70% of the simultaneously measured glomerular filtration rate. The possibility that protein binding made part of the streptomycin present in the plasma unavailable for filtration through the glomerulus was investigated. Two techniques were employed to measure protein binding. *1*) Measurement of the distribution of streptomycin on both sides of a cellophane membrane after dialysis of dog plasma against a Ringer solution containing varying amounts of streptomycin. *2*) Determination of streptomycin in the ultrafiltrate of plasma containing varying amounts of the drug. The dialysis experiments indicated 33 ± 3% and the ultrafiltration experiment 35 ± 4% binding of streptomycin to the plasma proteins of the dog. Within the range tested, 5 to 100 γ/ml., the percentage of streptomycin bound was independent of the concentration of the drug but dependent on the protein concentration. Both albumin and globulins are responsible for the binding. The observed protein binding accounts quantitatively for the observed clearance of streptomycin at about 30% below the simultaneously measured glomerular filtration rate.

Indirect *in vitro* action of antibiotics in comparison with activity of accepted amebacides. John L. Bradin, Jr., and Eder L. Hansen (introduced by Hamilton H. Anderson). *Division of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif.* A comparative study has been carried out which reveals differences in amebacidal levels between tests performed in cotton-stoppered versus vaseline-sealed cultures (see abstract by Hansen, *Federation Proc.*, this issue). As a base line for the antibiotic studies, the method has been applied also to retest representative amebacides, such as carbarsone, carbarsone oxide, emetine, vioform, diodoquin, and two thioarsenites (C.C. 914 and C.C. 1037). All antibiotics tested to date have shown some degree of bacteriostatic activity, and death of the ameba in cotton-stoppered tubes is shown to be due to inhibition of growth of the associated bacteria rather than to direct action of the antibiotic on *E. histolytica*. It has been shown previously (Bradin, in press) that death of the ameba, following exposure to an antibiotic which produced bacteriostasis, could be correlated with a shift in oxidation-reduction potentials of the cultures from negative to positive levels. Chang (1946) had reported that such positive levels constitute an environment lethal to this protozoan. It is, therefore, suggested that the indirect amebacidal activity, in the case of the antibiotics observed in cotton-stoppered cultures, is due, at least in part, to this phenomenon. Data are presented showing oxidation-reduction levels in cotton-stoppered and in vaseline-sealed cultures.

Inhibition of cholinesterase activity of human blood plasma. II. Studies with hexaethyltetrapolyphosphate containing C^{14}. Ralph W. Brauer and Rita L. Pessotti (by invitation). *Dept. of Pharmacology and Exper. Therapeutics, School of Medicine, Louisiana State Univ., New Orleans, La.* The mechanism of the interaction between plasma (cholin) esterase and neutral phosphate ester type inhibitors (e.g. hexaethyltetrapolyphosphate (HETP), Tetraethylpyrophosphate (TEPP)), has been studied in a previous paper (Ralph W. Brauer, *J. Pharmacol. & Exper. Therap.* 92: 162, 1948). Work to be reported represents an extention of that earlier investigation along the following lines: *1*) HETP containing C^{14} in the alkyl groups (alpha carbons) has been prepared. This material is being used to investigate whether the enzyme inhibition involves the fixation of any part of the inhibitor molecule upon the inhibited enzyme. The previous study had shown that the P containing moiety is not firmly attached to the enzyme, and that enzyme inhibition is paralleled by disappearance of inhibitor. *2*) The addition of F_2'' in amounts which do not affect the enzyme to aqueous solutions of HETP or TEPP markedly delays the loss of anticholinesterase activity, ob-

served under these conditions in the absence of fluoride. This reaction is described in detail, and discussed in relation to the enzyme-inhibitor reaction. 3) *In vivo* studies of the rate of recovery of liver and plasma cholinesterase activities of male rats after Di-isopropyl fluorophosphate TEPP and p-nitrophenyl thiophosphate administration are reported.

A single injection method for the assay of digitalis in pigeons. H. A. BRAUN and L. M. LUSKY (by invitation). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* A comparative study of digitalis assay has been made using a single-injection pigeon method, the USP XIII intravenous infusion cat method, and the intravenous infusion pigeon method (*J. Pharmacol. & Exper. Therap.* 93: 81, 1948). In the single injection method, digitalis dilutions were injected into the alar veins of pigeons at 3 dosage levels. Six pigeons were used on each dose. Death occured in from 3 minutes to 1 hour. The slope of the dosage mortality curve is very steep (on the order of 40 probits/log dose) and as a result the standard error of the assay is small. This method results in a considerable saving in time over the infusion methods. Assays by the 3 methods have been completed on a number of digitalis preparations of various types with good agreement between methods.

Evaluation of several types of drugs and combinations against anaphylaxis in guinea pigs. BARBARA B. BROWN (by invitation) and HAROLD W. WERNER. *Pharmacology Dept., Research Laboratories, Wm. S. Merrell Co., Cincinnati, Ohio.* Eight compounds possessing parasympathetic depressant, sympathomimetic, or direct smooth muscle depressant action were evaluated for antianaphylactic activity in guinea pigs actively sensitized to horse serum. Activity was determined following intraperitoneal injections of each compound and of combinations of each with a maximal noneffective dose of the antihistamine, Decapryn Succinate. The direct smooth muscle depressants, papaverine and Butaphyllamine (theophylline isobutanolamine), considered as a group were the most effective antianaphylactic agents both alone and in combination with Decapryn. Two of the parasympathetic depressants, atropine and Diocyl (diethylaminoethyl 1-cyclohexylcyclohexanecarboxylate), failed to protect against anaphylactic death, but doses of 6 to 14 mg/kg. injected with the noneffective dose of Decapryn reduced mortality by about 60%. A new parasympathetic depressant which also has direct smooth muscle depressant action, 2,2-diphenyl-4-dimethylaminomethyl-1,3-dioxolane methobromide, was ineffective alone but reduced mortality to a minimum when combined with Decapryn. Two sympathomimetic amines, Nethamine (*N*-ethylephedrine) and *N*-methyl-*N*-cinnamyl-2-phenylpropylamine, had no action alone but caused a significant reduction in mortality when employed in 6 to 17 mg/kg. doses with the antihistamine. A test of one triple combination composed of noneffective doses of Butaphyllamine, Nethamine, and Decapryn demonstrated marked potentiation of action. These results indicate that certain combinations of drug actions may prove valuable in allergic bronchial asthma.

Adrenergic-like action of acetylcholine on the isolated rabbit heart. H. D. BRUNER and KWANG SOO LEE (by invitation). *Dept of Pharmacology, Univ. of North Carolina, Chapel Hill, N. C.* The apparent adrenergic action of acetylcholine on atropinized isolated mammalian hearts has been further analyzed by adding tetraethylammonium Br, nicotine, d-tubocurarine, F-933, dihydroergocornine, ergotamine, dibenamine and priscol to the isolated rabbit heart perfused by the Langendorff technique. The findings throughout confirm the possibility suggested by Hoffman *et al.* (*Am. J. Physiol.* 144: 189, 1945) that there are ganglion cells on these hearts whose activities are to be classed as adrenergic. The dose of acetylcholine necessary to produce the adrenergic effect is some 10 to 100 times that to produce an intense typical cholinergic effect. No evidence has been found that acetylcholine has a direct myotropic action on ventricular muscle. Acetyl-β-methyl choline does not elicit the response.

Oral Activity of Analogues of 3-ortho-toloxy-1,2-propanediol. JOHN C. BURKE, RICHARD K. THOMS, G. LEE HASSERT, JR. and RITA J. WILKINS (introduced by CHARLES R. LINEGAR). *Pharmacological Development Division, E. R. Squibb & Sons, New Brunswick, N. J.* In June 1948 Berger and Schwarz (*J.A.M.A.* 137: 772.) reported that 3-ortho-toloxyl-1,2-propanediol (Tolserol) is effective by oral administration for the relief of various spastic conditions. One of the chief limitations to such use is its short duration of action. In an attempt to overcome this difficulty, a large series of compounds, synthesized and screened in Squibb Institute for Medical Research (*Tr. New York Acad. Sci.* 2 11, 2-5, 1948) for possible intravenous use, were reviewed. Seven of these compounds were selected on the basis of their intraperitoneal potency in mice and their solubility and screened for their depressant action orally in mice. Compounds with lower solubility than Tolserol showed for the most part a slower onset and longer duration of action. Solutions of Tolserol have as much as three times the activity as high concentration suspensions but a shorter duration of action. Two compounds of the series, 2-o-toloxyethanol and 3-thymyloxy-1,2-propanediol, showed the most advantageous combination of oral potency and duration of action in mice. The former compound proved irritant and emetic to dogs. Blood

level studies in dogs showed that the absorption of the latter compound was delayed but more prolonged and constant than that of Tolserol.

Trichloroethanol as a metabolic product of trichloroethylene. THOMAS C. BUTLER. *Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins Univ., Baltimore, Md.* When urine from dogs that have inhaled trichloroethylene vapor is acidified and heated, trichloroethanol is formed, presumably by hydrolysis of the glucuronide, urochloralic acid. Trichloroethanol is produced in larger amounts from trichloroethylene by the dog than is trichloroacetic acid, a metabolic product previously identified (Barrett and Johnston, *J. Biol. Chem.* 127: 765, 1939; Powell, *Brit. J. Indus. Med.* 2: 142, 1945). The immediate precursor both of trichloroethanol and of trichloroacetic acid may be chloral hydrate.

Influence of alpha-naphthylthiourea (ANTU) on iodine metabolism. RICHARD U. BYERRUM (by invitation), KENNETH W. COCHRAN (by invitation), and KENNETH P. DUBOIS. *Univ. of Chicago Toxicity Laboratory and the Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* Previous work in this laboratory has shown that the administration of iodide will protect rats against several lethal doses of ANTU while thyroidectomized rats are not protected by iodide. We have, therefore, studied the effect of acute ANTU poisoning on iodine metabolism. Thyroids of normal rats fed a synthetic diet had an average iodine concentration of 129 mg.%. Five hr. after a lethal dose (10 mg/kg.) of ANTU there was a 50% decrease in thyroid iodine. Twenty-four hr. after a sublethal dose (4 mg/kg.) of ANTU thyroid iodine was one-third the normal level. When normal rats were fed a diet containing 5.3 mg. I/gm. of diet for 2, 5, and 10 days the average iodine values increased to 201, 270, and 297 mg.% respectively for the 3 feeding periods. Various tissues from thyroidectomized rats contained as much iodine as those from normal rats after similar iodide feeding periods. Guinea pigs which have about 1/3 the thyroid iodine concentration of rats exhibited an increase in thyro idiodine and a decrease in thyroid weight with an overall loss in total iodine after 200 mg/kg. of ANTU. Feeding iodide failed to protect guinea pigs against ANTU and failed to increase the thyroid iodine concentration. ANTU inhibited the uptake of I^{131} by rat and guinea pig thyroids. Doses of 1 and 10 mg/kg. of ANTU to rats caused respectively 50% and 90% inhibition and 150 mg/kg. to guinea pigs caused 95% inhibition of thyroid uptake of I^{131}.

Relation of chemical structure to action of hyaluronidase inhibitor. B. CALESNICK and K. R. BUETNER (introduced by R. BEUTNER). *Dept. of Pharmacology, Hahnemann Medical College and Keeley Inst., Dwight, Ill.* A search was undertaken for chemically related substances which inhibit hyaluronidase. For testing hyaluronidase activity, we used the turbidimetric method by Kass and Seastone which is based on the precipitation of acidified horse serum by potassium hyaluronate. Hyaluronate and hyaluronidase were incubated with or without the substance investigated. The decomposition of hyaluronate was then tested by adding horse serum. Phenol proved to be active: 1 mg. inactivating 7.5 gammas of hyaluronidase; 1 mg. of cresol, 24 gammas; ortho cresol, 37.5 gammas per mg.; chlorine substitution products more. With longer side chains attached to phenol, the activity rises higher: 1 mg. of diamyl phenol inactivates 600 gammas (0.6 mg.) and hexylresorcinol, 400 gammas (0.4 mg.) while the unsubstituted resorcinol is close to phenol in activity. In contrast salicylic acid proved to be only feebly inhibiting, 1 mg. inhibiting only 5.0 gamma of hyaluronidase. These drugs were added to the testing mixture dissolved in alcohol which has no anti-enzymatic effect in the amounts used. The therapeutic use of massive doses of sodium salicylate in rheumatic fever may be explained by its low inhibiting power. Sodium gentisate, a metabolic product of salicylate, inhibits even less. Many anti-histamine drugs are known to restrict the spreading of a subcutaneously injected dye after hyaluronidase, but we found that these drugs do not inhibit hyaluronidase *in vitro*. Also, epinephrine which restricts spreading, does not inhibit hyaluronidase but enhances it.

Method for assaying potential coronary vasodilators. A. CAMERON (by invitation), E. SORENSEN (by invitation) and B. CRAVER. *Division of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.* In the Langendorff preparation of the perfused mammalian heart, primary effects upon the coronary circulation may be difficult to distinguish from effects secondary to changes in rate or the amplitude of the contractions. In studying a series of mono and di-alkyl amino derivatives of histamine, it was found that a better appraisal of the effects of these derivatives upon the actual coronary circulation could be achieved by perfusing the heart with a recycled perfusion fluid to which one microgram/ml. of 2-(Beta-naphthyl) imidazoline hydrochloride (Privine) had been added. The addition of 0.035–0.1 I.U/ml. of Pituitrin served almost as well but was somewhat less consistent in its action. This usually allowed the progressive development of a decreased coronary flow which only after many minutes adversely affected the rate and amplitude of the myocardial contractions. The addition of histamine or various derivatives of histamine to the perfusion fluid containing the Privine promptly led to a marked increase in the coronary flow, soon thereafter to an increase in rate, and finally to an

increase in the amplitude of the myocardial contractions. This order of changes bespeaks therefore a direct effect of the drugs upon the coronary circulation. This argument is strengthened by the effects of Pituitrin itself upon the isolated heart, since low doses can exert a considerable positive inotropic action, especially upon the hearts from younger animals with a presumed greater reserve of coronary flow. This balancing of two opposed effects has also been observed with Privine.

Arsenic content of normal hair in the Chicago area. W. J. R. CAMP and V. A. GANT (by invitation). *Dept. of Toxicology, Univ. of Illinois, Chicago, Ill.* Because arsenic effects symptoms mimicing those of many pathological conditions physicians frequently consider arsenic intoxication in differential diagnosis. Arsenic determination was requested in 18.5% of our cases for the year 1948. Hair is commonly submitted for analysis since it is simpler to collect than a 24-hr. urine specimen. In order to establish the amount of arsenic found in the hair of healthy normal individuals in the Chicago area, a series of 43 samples were analyzed by the Gutzeit method with an average of 0.51 μg. of arsenic calculated as arsenic trioxide and a range of 0.25 to 0.88 μg/gm. of hair. While these figures are somewhat lower than those of Künkele and Fordyce, Rosen and Myers, they agree reasonably well with those reported by Glaister. Since there is no way to differentiate 'interior' and 'exterior' arsenic the amount normally found will probably vary from area to area depending on the dietary habits of the community, the amount of smoke and soot in the atmosphere, etc. We did not find any clear cut difference with age (6–52 yr.), nor between smokers and non-smokers, nor between those who did and did not eat sprayed fruit.

Maintenance of *T. equiperdum* in vitro at 38 C. WILLIAM CANTRELL (introduced by E. M. K. GEILING). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* A modification of the technique of Geiman, Anfinson, McKee, Ormsbee and Ball for the cultivation of malaria parasites was used to maintain *Trypanosoma equiperdum in vitro* at 38 C. An increase in numbers of about 50% was observed during the first 4 hours when 40 mg./l. ascorbic acid and 160 mg./l. glutathione were added to the perfusion medium which contained casein hydrolysate, glucose, pyridoxine, nicotinic acid, nicotinamide, riboflavin, thiamin, calcium pantothenate, choline, and the inorganic salts of the medium of Geiman and coworkers. The trypanosomes were maintained in 14 ml. defibrinated rat blood as both citrate and heparin had an adverse effect. The blood was placed in a glass tube 5 cm. in diameter and 10 cm. long with one end covered with cellophane and suspended in 250 ml. of the perfusion medium. Small glass covered magnets in the blood phase and in the perfusion medium were used to promote diffusion through the membrane and to keep the trypanosomes in suspension. The medium was sterilized by filtration.

Comparative studies of dihydroergot derivatives. R. T. CAPPS (by invitation), H. M. SUCKLE (by invitation) and O. SIDNEY ORTH. *Depts. of Pharmacology and Neurosurgery, Univ. of Wisconsin Medical School, Madison, Wis.* Alterations of some of the pharmacological properties of the ergot alkaloids due to hydrogenation have been reported for dihydro derivatives of ergotamine and ergocornine. The present study is concerned with 2 additional hydrogenated derivatives, viz., dihydroergocristine (D.C.S. 90)- and dihydroergokryptine (D.H.K. 135)-Sandoz. Comparisons have been made of the 4 derivatives for both acute and chronic toxic quantities; pathological changes in the heart, lung, liver, spleen, and kidney; and pharmacodynamic actions on the cardiovascular system and gastrointestinal tract. Protection was found against cardiac irregularities which occurred spontaneously during the administration of various anesthetic agents or were initiated by epinephrine injections. Augmentation of movement of the gastrointestinal tract was observed.

Cyclic and noncyclic hydrocarbons and cardiac automaticity. C. JELLEFF CARR and JOHN C. KRANTZ, JR. *Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore, Md.* The inhalation of 12 different hydrocarbons by the dog was found to produce a sensitization of the myocardium to epinephrine. The compounds studied were ethane, propane, propylene, cyclopropane, n-butane, isobutane, *cis*-trans-butene-2, cyclobutene, cyclobutane, cyclopentane, isopentane, and 2,2-dimethyl-butane. Ethyelene inhalation did not sensitize the myocardium under similar experimental conditions. In an attempt to explore further this unique character of ethylene, additional compounds have been tried, including methylene cyclobutene, methylcyclobutane, cyclopentene, spirocyclopropane, ethylene oxide, propylene oxide and acetylene. Mixtures of cyclopropane and ethylene were found to produce a sensitization of the dog's myocardium in a manner similar to that observed with pure cyclopropane.

Curariform action of decamethylene-1,10-bistrimethylammonium bromide. JULIO C. CASTILLO (by invitation) and EDWIN J. DE BEER. *Wellcome Research Laboratories, Tuckahoe, N. Y.* Confirming the findings of Barlow and Ing, and Paton and Zaimis (*Nature* 161: 718, 1948) on the curariform action of polymethylene bisquarternary ammonium salts of the general formula $(CH_3)_3N\cdot(CH_2)_n\cdot N(CH_3)_3^{++}\cdot 2BR^-$, the decamethylene ($C_{10}$) member of the series has been found to have a curare-like activity exceeding that of d-tubocurarine chloride (d.t.c.), the potency varying with the species and

the method of test. It is less toxic and it causes less embarrassment of the respiration than d.t.c. In cats and dogs the intravenous injection of a curarizing dose of C_{10} causes no significant change in blood pressure unless a big dose sufficient to depress the respiration is given, in which case a typical asphyxial rise in blood pressure is observed. The curare-like action of C_{10} is not antagonized by prostigmine in doses adequate to antagonize the effect of d.t.c., but in certain cases appears to be antagonized by the C_5 member of the series. An interesting finding is the fact that if a dose of C_{10} is preceeded by a dose of d-tubocurarine chloride, the curarizing action of C_{10} is markedly inhibited.

N-(p-arsenoso-benzyl)-glycineamide HCl) as an amebacide, *in vitro* and *in vivo* in macaques. Y. T. CHANG and R. K. REED (introduced by HAMILTON H. ANDERSON). *Division of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif.* The substituted phenyl arsine oxide (NU-1067 supplied by Dr. E. L. Sevringhaus) contains 26% arsenic. It occurs as a stable white powder and is reportedly less toxic for mice, rats, and rabbits than oxophenarsine HCl. It has shown activity against trypanosomes, treponema, and borrelia. Since it is stable in solution, in contrast to many arsenoso compounds, a comparison of its amebacidal activity was made with mapharsen. *E. histolytica*, grown in egg slope medium, after 48 hours' exposure at 37°C. (with a single bacterial associate, organism '*l*'), was killed by mapharsen at 1:40,000. Nu-1067 was active within the range of emetine HCl (1:20,000). Marpharsen was active in monkeys only during the period of therapy, when given in toxic amounts (Anderson and Hansen, 1947). NU-1067 was administered at various dose levels to 11 macaques. Two received 25 and 50 mg/kg., intravenously, total for 5 days without clearance of *E. histolytica*. Two were given orally 30 and 100 mg/kg. total for 10 days with clearance of amebas for 6 weeks at the higher dose level. Two monkeys, similarly treated with 300 mg/kg., were cleared of amebas during 3 months' follow-up in one. One developed edema of the genitalia and increased urea-N. (20 mg.%) as well as depressed 'T' waves on the electrocardiogram. At 450 mg/kg., similarly administered, 2 were cleared over 3 months. At 650 mg/kg., similarly given, one was cleared. The other received identical treatment, had increased urea-N. (32.5 mg.%) and depressed 'T' waves also. At 1.0 gm/kg. orally for 10 days, the urea-N. after therapy was 20 mg.%. Thus, NU-1067, like mapharsen, was amebacidal *in vitro*, but in macaques amounts that cleared natural infections were within the toxic range.

Action of diphenhydramine (benadryl) in reversing the epinephrine reversal of blood pressure. GRAHAM CHEN and DAVID RUSSELL (by invitation). *Research Laboratories, Parke, Davis and Co., Detroit, Mich.* Diphenhydramine, 5 to 10 mg/kg., was found to reverse the 'epinephrine reversal' of blood pressure of a dog, previously receiving an adrenergic blocking agent. A number of the para-substituted derivatives of diphenhydramine and cocaine in larger doses can produce the same effect. Since these compounds also potentiate the pressor effect of epinephrine, this and the action in reversing the 'epinephrine reversal' of blood pressure may be similar in nature. The blocking of the vasodepressor receptors is suggested as a possible mode of action of these compounds on the vasopressor response to epinephrine.

Comparison of the cardiac action of thevebioside and neriifolin with thevetin. K. K. CHEN, ROBERT C. ANDERSON (by invitation), and FRANCIS G. HENDERSON (by invitation). *The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind.* Since the isolation of thevetin in our laboratory from the nuts of *Thevetia neriifolia*, a newer glycoside, neriifolin, has been independently obtained by Frèrejacque (*Compt. rend.* 221: 645, 1945) and Helfenberger and Reichstein (*Helvet. Chim. Acta* 31: 1470, 1948). By careful hydrolysis of thevetin with strophanthobiase, the latter succeeded in splitting 2 molecules of glucose, one by one, and separating in crystalline form 2 simpler glycosides, thevebioside and neriifolin. The last was identical with that occurring in nature. The digitalis-like action of either thevebioside or neriifolin was easily demonstrated by the systolic standstill of the frog's ventricle, and the characteristic changes of the cat's electrocardiogram. By comparison with thevetin, neriifolin was found to be about 4½ times as active in cats, and 3 times as active in frogs, as shown in the table. Thevebioside showed a higher potency in frogs, but was not much different from thevetin in cats. The median emetic doses were determined in both cats and pigeons.

	Thevetin	Thevebioside	Neriifolin
Cat Mean LD $\mu g/kg$	889.0 ±31.6	1004.0 ±103.8	196.1 ±10.3
Frog SD_{50} $\mu g/g$.	6.35 ± 0.26	3.78 ± 0.57	2.08 ± 0.19
Cat EmD_{50} $\mu g/kg$	300.0	161.0	64.7
Pigeon EmD_{50} $\mu g/kg$	211.8 ±13.8	141.0 ± 19.0	110.6 ±11.6

Prevention of fluoroacetate poisoning by acetate donors. MAYNARD B. CHENOWETH, EDWARD B. SCOTT (by invitation) and S. LOUISE SEKI (by invitation). *Dept. of Pharmacology, Univ. of Michigan Medical School, Ann Arbor, Mich.* The depression of contractility of isolated, perfused rabbit hearts or intestine induced by sodium fluoroacetate (FA) can be prevented by prior addition of sodium acetate. However, little or no protection of the intact rabbit can be obtained with even very large doses of sodium acetate. Administration of acetate

as monoacetin in doses of 2-4 gm/kg. one-half hour before a certainly lethal dose of FA (0.5 mg/kg.) prevented any symptoms of FA poisoning in 27 of 36 rabbits, while of 26 rabbits receiving the same dose of FA, 26 died. Alkalosis induced by sodium bicarbonate, fumarate or hydroxide greatly shortens the time to death of FA poisoned rabbits, probably explaining failures with sodium acetate. Five dogs receiving 0.1 mg/kg. FA died in an average of 330 minutes while of 14 dogs receiving a total of 5 gm/kg. monoacetin before and after the same FA dose, 5 survived without showing symptoms of FA poisoning. Average time to death of remainder of this group was 600 minutes. Monoacetin administration during the latent period characteristic of FA, or acute stages of poisoning, is without much effect. Monoacetin appears to be specific against FA and its congeners. That it acts by delivering a useful acetate moietie is indicated by the fact that it is equally or more effective than equimolar amounts of sodium acetate in increasing the acetylation of PABA by the rabbit. Some other acetate donors appear to be effective FA antagonists and will be reported upon.

Effect of local anesthetics on the motility of isolated rabbit ileum. GEORGE P. CHILD (introduced by ROBERT A. WOODBURY). *Dept. of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.* The spasmolytic action of a number of local anesthetics was studied using segments of isolated rabbit ileum. Most of the tests were made with Nupercaine, Pontocaine, Butacaine, Larocaine and Procaine. With the exception of Procaine these drugs decrease the normal motility of the ileum and relieve the spasm induced by Acetyl Choline and Barium Chloride. Their effectiveness on the intestine parallels their action as local anesthetics. 0.02% Procaine increases the amplitude of contractions; 0.04% increases amplitude and tone; 0.06% decreases the amplitude with increased tone; 0.1% decreases both amplitude and tone, acting like the other local anesthetics. The stimulating action of Procaine is not inhibited by Atropine. The action of these drugs appears to be neurotropic and musculotropic and is completely reversible on changing the bath. The question as to whether part of their effect is due to their action as local anesthetics is difficult to answer. Many synthetic antispasmodics also possess local anesthetic properties. The rapidity with which they often act in relieving the pain of peptic ulcer suggests that their success may be due in part to this local anesthetic action. Preliminary clinical trials with the local anesthetics indicate that they are effective in relieving pylorospasm, cardiospasm and the pain of peptic ulcer.

Action of synthetic antispasmodics and local anaesthetics on human gastric motility. GEORGE P. CHILD (by invitation) and ROBERT A. WOODBURY. *Dept. of Physiology and Pharmacology, Albany Medical College, Albany, N. Y. and Dept. of Pharmacology, Univ. of Tennessee Medical College, Knoxville, Tenn.* The synthetic antispasmodic, diethylamino ethyl phenyl thienyl acetate, Asymatrine, was found to be very useful in the relief of cardiospasm, pylorospasm, the pain of peptic ulcer and other gastro-enteric conditions. Some patients, however, voluntered the information that Asymatrine often produced a painless increased gastric motility. The action of Asymatrine and other antispasmodics on the motility of the human stomach was studied with intragastric balloons. Using Urecholine as the spasmogenic drug, Atropine, Homatropine, Pavatrine, Trasentin and Syntropan were spasmolytic in that order. Asymatrine, however, sometimes increased and sometimes decreased the motility. A comparison of the spasmolytic action of these drugs using isolated rabbit ileum with Barium Chloride and Acetyl Choline as spasmogenic agents, showed that Asymatrine was better than the others against Barium but not as good as Atropine or Homatropine against Acetyl Choline. A study of the local anesthetic action of these compounds showed that Asymatrine produced the best anesthesia of the rabbit cornea. On the basis of these apparently paradoxical results and a study of the spasmolytic action of a series of local anesthetics, Procaine, Butacaine, Larocaine, Pontocaine and Nupercaine were tried clinically for the relief of the pain of peptic ulcer, pylorospasm and cardiospasm with gratifying results. It is suggested that local anaesthetics be given a clinical trial in other conditions for which antispasmodics have been used.

Toxicology and pharmacology of lupulon. YIN-CH'ANG CHIN (by invitation) and HAMILTON H. ANDERSON. *Division of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif.* Lupulon, an antibiotic derived from hops (*Humulus lupulus*, L.) has been reported to be active against experimental mouse infections of *Mycobacterium tuberculosis* (Chin, Y. C., *et al.*, *Proc. Soc. Exp. Med.*, in press). The results so far obtained in toxicologic and pharmacologic studies follow: Intragastric LD_{50}'s in mice, rats and guinea pigs were 1500, 1800 and 130 mg/kg., respectively. The intramuscular LD_{50} was 600 mg/kg. for mice and 330 mg/kg. for rats. Convulsions were observed in animals which received lethal or sub-lethal intragastric or intramuscular doses Daily intragastric administration of lupulon for 12–14 days resulted in a slight suppression of growth of young rats which received 450 and 300 mg/kg., but not in those given 150 mg. A slight decrease in weight of rabbits occurred after 300 mg/kg. daily, and an increase of weight occurred in guinea pigs after 30 mg/kg/day over 14 days. Hemoglobin, RBC and WBC determinations in these ani-

mals were within the normal range. Intramuscular doses of 60 mg/kg/day for 30 days were tolerated without gross effects in mice. Lupulon, 4% added to diet, caused deaths in 50% of mice within 18 days, while 75 and 90% survived 40 days on food which contained 2 and 1%, respectively. All mice lost weight, which was less with lower doses. Neither hemolysis of rabbit erythrocytes nor loss of motility of leukocytes occurred *in vitro* within the effective antibiotic range. Respiratory stimulation was observed following intravenous injection of lupulon in both anesthetized and unanesthetized animals.

Ganglionic blocking action of diethylaminoethanol. BYRON B. CLARK and MAURICE M. HELPERN (by invitation). *Dept. of Pharmacology, Tufts College Medical School, Boston, Mass.* Recent quantitative studies show that diethylaminoethanol (DEAE) is rapidly formed after the intravenous injection of procaine, and that DEAE has therapeutic actions in man similar to procaine intravenously, with less toxicity (Brodie and coworkers, unpublished). Evidence for a ganglionic blocking action was obtained from the following observations on pentobarbitalized dogs and cats. DEAE-HCl injected intravenously in 30–60 seconds produces a prompt vasodepression of about 50% lasting 5–45 minutes with doses of 28 to 212 mg/kg. In the cat the inhibition of the contraction of the nictitating membrane to preganglionic stimulation paralleled the vasodepression, while postganglionic stimulation remained fully effective. The pressor response to splanchnic nerve stimulation was inhibited during DEAE vasodepression in adrenalectomized dogs, but typical pressor responses were obtained with the adrenals intact, and with injected epinephrine. Atropinization did not alter the vasodepressor response to DEAE. The heart rate is decreased about 10–25% during the vasodepression, and the vasodepressor and chronotropic effects on the heart of vagal stimulation are inhibited. In addition, the chronotropic effect of epinephrine on the heart appears to be inhibited. (DEAE has been shown to protect against epinephrine-cyclopropane ventricular tachycardia in dogs—Brodie.) DEAE has a positive inotropic effect on the isolated rabbit heart. On isolated intestine and uterus DEAE was found to have no significant cholinergic, anticholinergic, antihistaminic activity, or spasmolytic action against barium chloride. It does not antagonize the inhibitory action of epinephrine on intestine, but may have an adrenolytic action in high concentrations on rabbit uterus. The present evidence indicates that a major action of DEAE is to block transmission at autonomic ganglia.

Effect of fluoroacetate on oxidative processes in frog muscle. DONALD A. CLARKE (by invitation), JAMES F. TOOLE (by invitation) and WALTER F. RIKER. *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* The exposure of intact frog sartorii to varying concentrations of methyl fluoroacetate (1080) results in the disappearance of the recovery heat associated with contraction; the initial heat as well as the mechanical response remains unaltered. This indicates that fluoroacetate interferes with oxidative recovery processes in contracting muscle. Concentrations of sodium or methyl fluoroacetate as high as 10^{-3} M are without effect on the oxygen consumption of resting muscle. In contrast, however, a fluoroacetate concentration of 10^{-3} M produces a profound depression of the excess oxygen consumption which is associated with the action of caffeine. This suggests that fluoroacetate, like azide and hydroxylamine, is capable of differentiating the resting from the activity oxidative processes. In contrast to azide it does not inhibit the cytochrome oxidase system. To define further the locus of action, various intermediates of the carbohydrate cycle were added to the caffeine stimulated muscles poisoned with fluoroacetate. The substances tested were: lactate, succinate, pyruvate, glucose, fumarate, acetate, ketoglutarate, tartrate, and ATP. Of these, only pyruvate significantly antagonized the effects of fluoroacetate. A concentration of 10^{-2} M sodium pyruvate restored completely the activity oxygen consumption of the fluoroacetate poisoned muscles.

Loss of As from tissues by volatilization. RANDOLPH CLEMENTS, J. EUGENE HILL and HERSHAL G. TREE (by invitation), P. L. EWING and G. A. EMERSON. *Dept. of Pharmacology, Univer. of Texas Medical Branch, Galveston, Texas.* As-balance studies were made with mice and rats treated repeatedly with oral doses of K arsenite, Fe arsenate and Fe arsenate plus kaolin, or with water and sludge samples from a Texas stream which had been heavily polluted with Na arsenite and then treated with $FeSO_4$ and lime. Recovery of As from animals analyzed immediately after oral or intraperitoneal treatment with any of these dosage forms was essentially quantitative by either the A O A C official Gutzeit or bromate method. On repeated treatment, the balance between the amount given and the amount recovered in the excreta plus tissue As left a deficit of 30–60%, depending upon dosage. Less than 1% of the 'lost' As was recovered from the air by bubbling through concentrated HNO_3, with a group of rats maintained in a bell jar for two weeks and given daily injections of 5 mg/kg. of AS_2O_3 as K arsenite. After absorption of inorganic As_3 the As must be in part converted into volatile substances which either are not absorbed by efficient bubbling through HNO_3 or are retained in the tissues and are volatilized on treatment with H_2SO_4 and HNO_3.

Toxicity of N-p-chlorophenyldiazothiourea

(promurit) to mammals. KENNETH W. COCHRAN (by invitation) and KENNETH P. DUBOIS. *Univ. of Chicago Toxicity Laboratory and the Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* N-p-chlorophenyldiazothiourea (Promurit) has been employed as a rodenticide in Germany. The chemical similarity of Promurit to alphanaphthylthiourea (ANTU) made it of interest to compare the toxicity and pharmacological effects of the two compounds. For injection propylene glycol solutions of Promurit were employed. The approximate LD_{50} values in mg/kg. after intraperitoneal injection were: female albino rats, 0.2; mice, 1.35; guinea pigs, 1.9; and rabbits, 1.75. The approximate LD_{50} for Promurit given orally to female rats was 0.28 mg/kg. The compound is, therefore, more toxic than ANTU. The outstanding pathological change in acutely poisoned animals was pleural effusion. Repeated doses of Promurit resulted in the acquisition of some tolerance. It was acceptable to rats and produced 100% mortality when offered overnight in the diet at concentrations as low as 0.05%. Some increase in blood glucose and a depletion of liver glycogen followed acute poisoning by Promurit. The rodenticide caused a transitory inhibition of tyrosinase and produced a 50% inhibition of peroxidase at a final concentration of 1×10^{-4} M. Feeding iodide to rats before the injection of Promurit resulted in a two-fold increase in resistance toward the rodenticide. Thiosorbitol was ineffective therapeutically against Promurit poisoning while thiourea given prior to Promurit protected against several times the lethal dose. The extremely high toxicity of Promurit and the lack of an antidote appear to limit its possible usefulness as a rodenticide.

Effect of epinephrine on toxicity of procaine. VERSA V. COLE and H. R. HULPIEU. *Dept. of Biochemistry and Pharmacology, Indiana University, Indianapolis, Ind.* All drugs were given intravenously on a per kg. basis. Dogs anesthetized 45 minutes with diethyl ether were infused continuously with 2 mg/min. procaine hydrochloride until death. There were 4 groups of 10–12 dogs each as follows: *I*-controls, *II*-3 micrograms epinephrine every 5 minutes, *III*-3 μg/min. for the first 10 minutes of procaine, *IV*-2.5 μg/min. continuously with the procaine. Two other groups of 5 each anesthetized with thiopental sodium were given 3.5 mg/min. procaine hydrochloride until death. *Group V* were controls, *VI* received continuous epinephrine as *IV*. Survival time varied greatly, but averages in minutes were: *I*-21, *II*-19, *III*-17, *IV*-38, *V*-56, *VI*-44. The differences in time between respiratory failure and heart failure in minutes were: *I*-1.55, *II*-1.58, *III*-1.25, *IV*-0.65, *V*-2.0, *VI*-1.1. The blood levels of procaine in mg. % at heart failure were: *I*-3.7, *II*-3.7, *III*-2.6, *IV*-4.4, *V*-10.7, and *VI*-7.7. The levels of paraamino benzoic acid in blood in mg. % were: *I*-0.74, *II*-0.70, *III*-0.42, *IV*-*IV*-1.54, *V*-4.9, and *VI*-4.5. Continuously administered epinephrine in the dose given has a favorable effect on resistance to procaine under ether anesthesia. This effect is not observed under anesthesia with thiopental.

Tetraethyl ammonium and the response of the isolated rabbit heart to nicotine. J. M. COON, P. R. SALERNO (by invitation), and L. E. ELLINWOOD (by invitation). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* The isolated rabbit heart gives a diphasic response to a single treatment with 1 mg. of perfused nicotine. A complete standstill of about 1 minute duration ceases abruptly to be followed by a marked acceleration and an increase in amplitude lasting about five minutes. Smaller doses of nicotine produce only acceleration. Larger doses elicit a longer period of standstill followed by a more intense stimulation. It is thought that the depression is due to a stimulation of parasympathetic ganglia and that the acceleration may be due to axon reflexes induced in the postganglionic sympathetic innervation. If this were true tetraethyl ammonium (TEA) might be expected to abolish both phases of the cardiac response to nicotine. Perfusion of the isolated heart with 6×10^{-5} M TEA for 10 minutes abolished the inhibitor but not the accelerator response to nicotine. However, 20 minutes perfusion with this concentration of TEA also abolished the accelerator response. Discontinuation of the TEA perfusion was followed by a rapid recovery of the accelerator response, while the inhibitory effect returned more slowly upon washing out with Ringer-Locke solution. If the mechanism of the nicotine action is as described above these findings indicate that the threshold for parasympathetic ganglionic stimulation is higher than that for postganglionic sympathetic fiber stimulation. This would explain the pure cardiac acceleration elicited by small doses of nicotine. Furthermore, the TEA paralysis of the sympathetic fibers appears to be more readily reversible than that of the parasympathetic ganglia.

Comparative activities of five antispasmodics. B. CRAVER, W. BARRETT (by invitation), A. CAMERON (by invitation), A. EARL (by invitation), F. ROTH (by invitation). *Division of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.* The activities of 3 known antispasmodics: Trasentine, Trasentine-6H and atropine were compared with those of two new drugs, N-dimethyl thymoxy acetamidine hydrochloride (Su-198), and Thymoxyethyldiethylamidine hydrochloride (Su-216) in respect to the functions noted. They were examined in respect to the dose in μg/kg. required to inhibit salivation in the cat induced: *1*) by histamine; *2*) by pilocarpine, and *3*) by faradization of the chorda tympani nerve. In order of decreasing activity they were: atropine,

Trasentine-6H, Su-198, Su-216 and Trasentine. For each of the 6 drugs, the doses required were essentially the same irrespective of the sialogogic stimulant. The same doses of each drug that inhibited salivation eliminated the spasm of the nictitating membrane induced by subcutaneously injected pilocarpine. To eliminate the hypotension induced by 0.5 y/kg. of Mecholyl, approximately a 10-fold increase in the dose of each drug (compared to anti-sialogogic doses) was required. This relationship was strictly quantitative and larger doses of Mecholyl produced hypotension. The order of spasmolytic activity of the 5 drugs remained the same in respect to this function as well as in respect to their ability to eliminate the brief bradycardia and hypotension induced by faradization of the peripheral ends of the severed vagi, but for this latter function only about 5 times the anti-sialogogic doses were needed. The respective LD_{50}'s (I.V. rats) for the 5 drugs in mg/kg. are: Su-216—65, Su-198—45, Trasentine-6H—c.37, Trasentine—c.27, and atropine—c.100–125. The problem of attempting to predict the therapeutic usefulness of antispasmodics will be discussed.

Efficacy and toxicity of simaroubidin in experimental amoebiasis. ASHTON C. CUCKLER and CARL C. SMITH (introduced by HANS MOLITOR). *Merck Institute for Therapeutic Research, Rahway, N. J.* A standardized procedure has been developed for the evaluation of potential amoebicides in young rats infected with *Endamoeba histolytica*. One of the compounds evaluated in this way, and which is more active therapeutically than many of the currently used amoebicides, is simaroubidin, a crystalline material isolated from the plant *Simarouba amara*. Single oral doses of 25 mg/kg. do not have significant amoebicidal activity, whereas single doses of 100 mg/kg. % CD_{90}. Single doses of 200 and 400 mg/kg. % CD_{100}. Six daily doses of 2 mg/kg. and 10 mg/kg. are ineffective, but doses of 50 mg/kg. have significant ($>CD_{50}$), although not complete, effectiveness. A drug diet of 0.01% given *ad libitum* for 6 days has only slight amoebicidal activity; drug diets of 0.04% and 0.16% % CD_{90}. The acute and chronic toxicities of simaroubidin have been determined for several species. The LD_{50} for rats and mice of single oral doses was 800 ± 100 mg/kg. In a dog single oral doses of 100 mg/kg. produced loss of appetite, but an oral dose of 400 mg/kg. was lethal in 2–3 days. Repeated daily oral doses of 500 mg/kg. were lethal for 9/10 rats within 10 days, and 250 mg/kg. killed 3/12 rats within 21 days. Doses of 50 mg/kg. for 21 days produced no mortality and only slight effect on growth of young rats. When the drug was placed in the diet at 0.16% and fed to rats for 21 days the growth was significantly depressed and 1/12 rats died, but no gross pathologic changes were observed at autopsy.

Comparative muscle-relaxing activity of seven glycerol ethers on isolated nerve-muscle preparations. HAROLD DAVIS (by invitation), CHARLES H. HINE and FRANK J. MURPHY (by invitation). *Division of Pharmacology and Experimental Therapeutics and Dept. of Anesthesiology, Univ. of California Medical School, San Francisco, Calif.* Two methods of producing muscular contractions have been adapted for *in vitro* comparison of muscle-relaxing agents. Contractions of frog recti abdomini, suspended in frog Ringer solution (Torda and Wolff, 1944), were recorded. The muscle was stimulated either by physostigmine (2×10^{-5}) plus acetylcholine (10^{-7}) or by tetanic current (100–200 V.). In each case the endpoint was arbitrarily designated as 50% reduction of normal contraction following addition of the compound. The duration of exposure to the solution was 30 minutes or less. This technic yielded consistent results. Seven modifications of glycerol ethers, procaine, and Intocostrin were tested. The greatest dilution of each compound which produced an endpoint are listed in the accompanying table. Since the results obtained *in vitro* paralleled those secured in mouse assay procedures, the *in vitro* technics may serve as a guide to possible muscle-relaxing activity *in vivo*.

Compound	AcCh-Eserinized Muscle Stimulation		Electrical Nerve-muscle Stimulation	
	Dilution V/V or W/V	Molarity mM /liter	Dilution V/V or W/V	Molarity mM /liter
Intocostrin (20 u/ml.)	10^{-3}	—	10^{-3}	—
Procaine HCl	5×10^{-4}	1.8	5×10^{-4}	1.8
Monocyclohexyl	10^{-4}	0.61	5×10^{-4}	3.0
a,γ-isopropyl ethyl	Contracture before endpoint was reached			
a,γ-isopropyl tolyl	10^{-5}	0.065	5×10^{-5}	0.33
Phenyl monoacetate	10^{-3}	5.5	5×10^{-4}	2.75
Phenyl diacetate	5×10^{-4}	2.3	5×10^{-4}	2.3
Dibutyl monoacetate	10^{-4}	0.4		
o-Toxyl(Myanesin)	10^{-3}	5.5	5×10^{-4}	2.75

Antiepileptic action of Marihuana-active substances. JEAN P. DAVIS and H. H. RAMSEY (introduced by LOUIS S. GOODMAN). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah, and Utah State Training School, American Fork, Utah.* The demonstration of anticonvulsant activity of the tetrahydrocannabinol (THC) congeners by laboratory tests (Loewe and Goodman, *Federation Proc.* 6:352, 1947) prompted clinical trial in 5 institutionalized epileptic children. All of them had severe symptomatic grand mal epilepsy with mental retardation; three had cerebral palsy in addition. Electroencephalographic tracings were grossly abnormal in the entire group; three had focal seizure activity. Their attacks had been inadequately controlled on 0.13 gm. of phenobarbital daily, combined with 0.3 gm. of Dilantin per day in 2 of the patients, and in a 3rd, with 0.2 gm. of Mesantoin daily.

Two isomeric 3 (1,2-dimethyl heptyl) homologs

of THC were tested, Numbers 122 and 125A, with ataxia potencies 50 and 8 times, respectively, that of natural marihuana principles. Number 122 was given to 2 patients for 3 weeks and to 3 patients for 7 weeks. Three responded at least as well as to previous therapy; the 4th became almost completely, and the 5th, entirely seizure free. The optimum dosage was 1.2 to 1.8 mg. daily. One patient, transferred to 125A after 3 weeks, had prompt exacerbation of seizures during the ensuing 4 weeks, despite dosages up to 4 mg. daily. The 2nd patient transferred 125A was adequately controlled on this dosage, except for a brief period of paranoid behavior 3½ weeks later; similar episodes had occurred prior to cannabinol therapy. Other psychic disturbances or toxic reactions were not manifested during the periods of treatment. Blood counts were normal. The cannabinols herein reported deserve further trial in non-institutionalized epileptics.

Anti-anemic agents and cholinesterase activity. JOHN EMERSON DAVIS. *Pharmacological Laboratory, Univ. of Arkansas, Little Rock, Ark.* Considerable evidence is accumulating which indicates that an increase of cholinesterase activity may play an important part in the therapeutic remission of pernicious anemia induced by the administration of liver extract or folic acid. As a further contribution to this evidence, the author has tested the effects of liver extract and folic acid upon the cholinesterase activity of powdered liver (Viobin 40° liver). One mg. of pteroylglutamic acid or 0.1 unit of crude liver injection was added to a suspension of 10 mg. of powdered liver in 2 cc. of tap water. The mixture was incubated at 37° C. for 3 hr. with occasional agitation or shaking, and then its cholinesterase activity was measured by an electrometric titration method, previously described (*Proc. Soc. Exper. Biol. & Med.* 63: 287, 1946). When acetylcholine bromide was used as substrate, both liver extract and folic acid caused an average 7-fold increase in the initial hydrolysis rate produced by the powdered liver. When Mecholyl chloride was used as the substrate, folic acid increased the enzyme activity of the powdered liver by only about 4-fold while the liver extract increased the activity by 20-fold. Under the conditions of our experiment, it seems that liver extract causes an activation, or perhaps formation, of an enzyme which has some of the properties of a specific cholinesterase, while folic acid increases an enzyme activity which resembles that of a non-specific cholinesterase.

A fatal case of Bartonella Canis treated with penicillin. JOHN EMERSON DAVIS. *Pharmacological Laboratory, Univ. of Arkansas, Little Rock, Ark.* Since reports of Bartonella Canis infection in splenectomized dogs are quite rare, it seems pertinent to report a severe spontaneous case which showed many of the signs and symptoms of human Oroya Fever. About 90 days after splenectomy had been performed, one of our dogs appeared to be quite emaciated and was found to have a severe anemia. He had a low grade fever (102.3°), leukocytosis (23000), weakness and pain, a red cell count of 770,000, 2.5 gm. % of hemoglobin, a hematocrit of 10.5%, an icteric index of 112, and reticulocytosis (up to 40%). About 75% of the erythrocytes were parasitized by organisms which appeared to be identical with Bartonella Canis. Five cc. of blood from this animal was transferred into a second splenectomized dog which, 80 days later, was very anemic (1.9 million RBC) and had parasitzed cells, but slowly and spontaneously recovered. Penicillin was given to the original bartonella dog over a 3-day period (10,000 U. every 6 hr., I.M.). On the 3rd day of treatment, the blood was cleared of parasites, the icteric index was greatly reduced, and temperature and leukocyte count were normal. By the 4th day, the erythrocyte count had more than doubled, but on the 7th day parasites began to reappear in the blood, and the anemia subsequently became more severe. The dog died in coma, 26 days after his condition had been discovered.

Thiopental hypnosis in rabbits on experimental diets. B. DEBOER. *Dept. of Pharmacology, St. Louis Univ., School of Medicine, St. Louis, Mo.* The addition of $NaHCO_3$ to the diet of rabbits fed whole oats *ad libitum* was accompanied by a shortening of the sleeping time following 2 intravenous injections of 25 mg/kg. thiopental an hr. apart. Previous information (*Proc. Soc. Exper. Biol. & Med.* 68, 1948) indicated that the sleeping time of rabbits on a diet of oats for one month was 60% longer than that of rabbits on a diet of Purina rabbit chow. Maintaining these animals upon the experimental diet as long as 20 weeks did not significantly change the effects of thiopental beyond that occurring in 4 to 8 weeks. The addition of $NaHCO_3$ to the whole grain diet (1% in the daily water supply, which was sufficient to render the urine alkaline) reduced the sleeping time after one week to within 25% of the previous control time. Intravenous injection of 300 mg/kg. $NaHCO_3$ (3 cc. of a 10% solution/kg. of animal, resulting in approximately 1 gm/animal) preceding the administration of thiopental was without effect the duration of sleep. The effect of diet upon pentobarbital sleeping time was found to be similar. Animals fed rabbit chow averaged 36 and 66 minutes sleeping time after the first and second injections of 25 mg/kg. pentobarbital one hr. apart. Animals fed whole oats for one month showed only partial recovery during the first hour after the injection and slept an average of 185 minutes after the second injection given at the end of the first hr.

Pentobarbital hypnosis in guinea pigs on ascorbic acid-deficient diet. B. DEBOER and K. O. DE BOER (by invitation). *Dept. of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, Mo.* For

58 male and 21 female guinea pigs on a normal diet the sleeping time was 210 minutes when given 25 mg/kg. pentobarbital via the peritoneal cavity. The average weight of these animals was 480 grams. After 14 days upon a vitamin C deficient diet the duration of pentobarbital hypnosis was 50% longer than that of controls and after 21 days on the diet 65% longer. The weight of these animals decreased 19% after 14 days and 22% after 21 days on the deficient diet. Returning the animals to a normal diet for 21 days restored the weight to nearly normal and the duration of sleep to within 10% of the previous normal. Ascorbic acid 20 mg/kg. given intramuscularly immediately preceding the pentobarbital injection had no apparent effect on the duration of sleep of animals on the deficient diet. Ascorbic acid given 20 or 100 mg/kg. 1 hour prior to the injection of the barbiturate in animals 14 days upon the deficient diet had but little effect but in those 21 days upon the diet the sleeping time was 58% above the normal after 20 mg/kg. ascorbic acid and 42% above normal after 100 mg/kg. ascorbic acid instead of the 65% increase previously noted. In 9 animals on the deficient diet for 14 days a single injection of 100 mg/kg. of ascorbic acid was accompanied by a decrease in the duration of sleep (when tested 7 days later) to about 10% above the preexperimental control sleeping time.

Certain physico-chemical and toxic properties of phenol-camphor preparations. Wm. B. Deichmann and T. Miller (by invitation). *Division of Pharmacology, Dept. of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.* The solubility at 23°C. of phenol in mineral oil was found to be 0.1%, in aromatic mineral oil (mineral oil containing traces of eucalyptus and safrol) 0.33%. A 10° increase of temperature raised the solubility about 6 times, to 0.65 and to 1.87%, respectively. Addition of 40 ml. of water to 25 ml. of a saturated solution of phenol in mineral oil (in a 100 ml. separatory funnel having a diameter of 3.88 cm.), or to phenol in aromatic mineral oil brought about a redistribution of phenol. At equilibrium, the water phases contained 60 and 50% of phenol respectively. Similar equilibration of a solution of 4.75% phenol and 10.86% camphor in aromatic mineral oil against water led to a phenol concentration in the aqueous phase of 1.026%. Doubling the phenol in the camphor-mineral oil mixture also doubled its concentration in the aqueous phase. The lethal dosages (LD_{50}) of phenol solutions administered as single doses by stomach tube to albino rats were as follows in terms of phenol content: 0.5 gm/kg. when given as a preparation of 3.56% phenol in aromatic mineral oil (heated to 34°C), 0.72 gm/kg. as a solution of 4.75% phenol in water, and 1.06 gm/kg. as a 4.75% solution of phenol and 10.86% camphor in aromatic mineral oil. Immersion of the tails of albino rats in solutions containing various quantities of phenol continuously up to 8 hours, established the following concentrations that killed approximately 50% of one strain of albino rats: 2.0% phenol in aromatic mineral oil, 4.4% phenol in water, and 7.9% phenol plus 10.86% camphor in aromatic mineral oil. With another strain of albino rats the corresponding LD_{50} values were 2.7% phenol in aromatic mineral oil, 6.6% phenol in water, and 11.5% phenol plus 10.86% camphor in aromatic mineral oil.

Effects of epinephrine, ephedrine and 1-(m-hydroxyphenyl)-N²-methyethylene diamine (NU-1683) on the coronary blood oflw. Adam B. Denison, Jr. (by invitation), Ben J. Lawrence, Jr. (by invitation) and Harold D. Green. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.* Wayne *et al.* have reported at this meeting that 1 μgm of epinephrine, 1 mg. of ephedrine and 1 mg. of NU-1683 produce approximately at 25–50% reduction in flow in the hind leg when injected into the femoral artery. This paper presents the effects of intracoronary artery injections of similar amounts of these drugs upon the coronary blood flow. In dogs anesthetized with pentobarbital, the heart was exposed by removal of the anterior halves of the left 4th and 5th ribs. Heparin plus, in some experiments, chlorazol fast pink, served as anticoagulant. Flow of blood from a carotid into the anterior ramus descendens was measured with a Gregg-Green orifice meter and differential manometer. Epinephrine in the above dosage caused a 50% increase in flow at the end of diastole. The above doses of NU-1683 and ephedrine had no effect. With rise in arterial pressure, which frequently occurred as the drugs reached the systemic circulation, flow increased with all three drugs.

Biosynthesis of radioactive bufagin containing C^{14}. John Doull (by invitation), Kenneth P. DuBois, and E. M. K. Geiling. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* An investigation of the optimum conditions for the biosynthesis of radioactive bufagin with C^{14} was undertaken to obtain labeled bufagin for studying the distribution and fate of this cardiac glycoside in mammals. Since C^{14} as carbonate was rapidly excreted in the expired air it was more desirable to feed an organic form of carbon; algae (supplied by Dr. W. F. Libby) grown in the presence of C^{14} and containing 406 uc C^{14}/gm. were, therefore, employed. Tropical toads (*Bufo agua*) were used for the biosynthesis. They were kept in an enclosed system through which CO_2-free air was passed allowing the expired CO_2 to be collected in alkali and measured. Two weeks after feeding C^{14} the secretion was collected from the parotid glands and the animals were sacrificed for quantitative measurement of the C^{14} in the tissues. One toad receiving 50 uc C^{14} as radioactive algae contained 8.9% of the administered

dose in the tissues, 11.8% was exhaled as CO_2 and 79.3% was excreted in the urine and feces. Two weeks after 100 uc of C^{14} 29.4% of the original dose was present in the tissues, 17.9% was exhaled as CO_2 and 52.7% was excreted in the urine and feces, mainly in the feces. Of the C^{14} in the tissues the liver contained 16.9%, skeletal muscle and bones 60.0%, small intestine 10.4% and the remainder was rather evenly distributed throughout the other tissues. Radioactive bufagin and epinephrine were obtained from the parotid secretion.

Comparative percutaneous toxicity of 3-mercapto-1,2-propanediol (thioglycerol) and ammonium thioglycolate. JOHN H. DRAIZE, ELSIE ALVAREZ (by invitation) and MARIE WOODARD (by invitation). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Ammonium thioglycolate is used extensively in the 'cold-hair wave process.' To a lesser extent thioglycerol is similarly employed. The thioglycolate 'hair waving' procedure is sometimes varied by the application of heat. Ninety-day percutaneous toxicity experiments on the rabbit were undertaken with 7% ammonium thioglycolate (with solution at room temperature and also heated to 47°C), and with a 6% thioglycerol waving solution at room temperature. All waving solutions were adjusted to a *pH* 9.2 ± 0.1. Blood examinations, urinary sulfur excretion, and systemic effects in subjects were recorded. Histological examination of tissues were made. Sensitization studies were conducted on the guinea pig. When 4.0 ml/kg/day (a total of 65 doses) of the waving solutions were inuncted mortalities of 74, 61 and 0% were obtained with ammonium thioglycolate (47°C), ammonium thioglycolate (room temperature) and the thioglycerol respectively. The percentage increase of urinary sulfur reached peak figures of 147, 105 and 83 for ammounium thioglycolate (47°C), ammonium thioglycolate (room temperature) and thioglycerol respectively. Symptoms of poisoning in animals had a rapid onset with all agents and upon discontinuance of treatment recovery was prompt. Extentensive skin damage was always associated with serious poisoning. While thioglycerol showed less gross evidence of toxicity it produced a high incidence of sensitization and evidence of pathologic changes in the thyroid gland.

Experimental skin sensitization to sulfonamides and penicillin. ROBERT H. DREISBACH. *Dept. of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco, Calif.* Since both sulfonamides and penicillin have been suspected clinically of increasing skin sensitization to each other attempts were made to test this experimentally. Sensitization of the Arthus type was attempted in 12 rabbits by giving 6 weekly subcutaneous injections of 1 cc. 0.8% (saturated) sulfanilamide, or 0.06% (saturated) sulfathiazole, in 0.9% saline solution in separate groups of 6 rabbits each, controlled by similar injections of 0.9% saline. No noticeable swellings or reactions occurred. In order to test possible increased susceptibility to penicillin sensitization in the sulfonamide injected areas of these rabbits, a total of 4 injections of 10,000 units each of crystalline penicillin G in 1 cc. of saline solution were made at weekly intervals. Three of 11 rabbits showed a positive Arthus phenomenon, chiefly minor swellings at the injection sites. In our experience, as many as 10 of 12 normal rabbits may show sensitivity reactions to crystalline penicillin G alone. Following the penicillin injections, the same 12 rabbits were given 2 injections at weekly intervals of sulfanilamide or sulfathiazole into the penicillin-sulfonamide areas, in the same manner as before, but no reactions occurred. In another attempt to produce sensitization, cutaneous applications were made of 1% solutions of sulfanilamide or fathiazole in carbitol (known for epidermal penetration) at weekly intervals on separate groups of 6 rabbits each. No sensitization occurred after 8 applications, although the sulfonamides were absorbed (present in urine). According to these methods, therefore, sulfonamide treated skin is not demonstrably sensitized nor does such treatment increase penicillin sensitization.

Inhibitory action of bufagin on carbohydrate oxidation. KENNETH P. DUBOIS, JOHN DOULL (by invitation), and E. M. K. GEILING. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* In connection with studies on the mechanism of action of cardiac glycosides the effect of bufagin on enzymatic reactions was investigated. Bufagin was isolated from the parotid secretion of the tropical toad, *Bufo agua*, by the method of Jensen and Chen (*J. Biol. Chem.* 87 : 755, 1930). Since this glycoside has a very low solubility in aqueous media preliminary experiments were carried out by addition of the crystalline compound to achieve saturation of the test system. For further experiments 50% ethanol solutions of bufagin were prepared and further diluted with water for use; appropriate ethanol controls were included. Addition of crystalline bufagin to isotonic brain homogenates resulted in marked inhibition of the oxidation of glucose. The amount of inhibition was consistently greater than 65% for rat, guinea pig and mouse brain homogenates. Further tests showed that a final concentration of 2×10^{-4} M bufagin resulted in 50% inhibition of the respiration of isotonic brain homogenates. The respiration of brain slices was inhibited to about the same extent as homogenates by similar concentrations of the glycoside. The rate of oxidation of glucose by heart slices from guinea pigs, rats and mice was decreased about 75% by saturating the test system with bufagin. The oxidation of glucose by liver and kidney slices was only

inhibited to the extent of about 15% by saturation of the media with the drug. Bufagin had no effect on anaerobic glycolysis nor on the cytochrome oxidase and succinic dehydrogenase systems.

Bromate distribution in blood. A. L. DUNN (by invitation) and A. R. MCINTYRE. *Dept. of Physiology and Pharmacology, Univ. of Nebraska, College of Medicine, Omaha, Nebr.* The distribution and fate of ingested bromates has not been studied quantitatively previously. In these studies the distribution of bromate in the red cells and serum was determined following the administration of approximately ⅔ of the delayed M.L.D. per os in the dog. After 75 mg. of $KBrO_3$/kg. gram by stomach tube in 100 cc. of water followed by another 100 cc. of water, blood was collected from a jugular vein at intervals of ½ hour, 1 hour, 2 hours, 4 hours and 24 hours. No anesthetic and no anticoagulant were used. The blood was defibrinated, centrifuged, the serum drawn off, the red cells washed in isotonic saline and hemolyzed, and protein-free filtrates were then prepared. Bromate was determined by the decolorization of Evans blue. The decolorization of this dye has a marked specificity for bromate over the other halates, and was previously described by us (*J. Lab. & Clin. Med.*, in press). This method was modified in such a manner that the bromate was determined spectrophotometrically. The findings indicate that bromate in the above dosage is absorbed rapidly from the gastrointestinal tract and the peak concentration is found in the blood filtrate within one hour following ingestion. The concentration remains at a high level for 3 hours and falls to a negligible amount at 24 hours. No bromate was found in the saline-washed cells.

Renal clearance of streptomycin. A. O. EDISON (by invitation), V. C. JELINEK (by invitation) and G. E. BOXER. *Merck Institute for Therapeutic Research and The Research Laboratories of Merck & Co., Inc., Rahway, N. J.* The renal clearance of streptomycin in dogs was obtained by fluorometric determination of the drug in the plasma and urine. The clearance was measured in 5 experiments over 6 equal one-half-hour periods after a single intramuscular injection of the drug. The normal glomerular filtration was determined simultaneously by measuring the renal clearance of thiosulfate ions since the streptomycin interferes with the determination of inulin, mannitol and creatinine. The clearance of streptomycin was on the average 70% below the simultaneously determined glomerular filtration rate. Wide variations in urine flow and the concentration of the drug in the plasma did not influence the clearance, apparently excluding tubular reabsorption. The clearance measured from the rate of disappearance from the plasma and from the calculated volume of distribution of streptomycin was found to agree with the clearance obtained in the conventional manner from the concentration in plasma and urine and the rate of urine flow. The implications of this finding with respect to the volume of distribution and the manner of excretion are discussed. The clearance calculated from the plasma concentration of the drug was found to be identical for man and dog per unit of surface area.

Biliary excretion and intestinal reabsorption of C^{14}-labeled methadone hydrobromide. L. L. EISENBRANDT, I. A. ABDOU and T. K. ADLER (introduced by H. W. ELLIOTT). *Division of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif.* Using the standard technics of tracer investigations, C^{14} incorporated into methadone was administered subcutaneously into rats for the purpose of determining methadone excretion via the digestive tract. Elliott, *et al.* (*J. Pharmacol. & Exper. Therap.* In press) in their distribution studies showed relatively large amounts of radioactive methadone in the liver, intestine and in feces. In the present study the bile duct was cannulated and bile collected for the duration of analgetic effects which usually lasted about 2 hours following 1.5 mg. of methadone. After injection into the left hind leg, the bile became radioactive in approximately 10 minutes. The peak of radioactivity was reached in 60–90 minutes and decreased as analgetic effects diminished. Although much of the radioactive methadone was excreted in the feces, reabsorption in the small intestine appeared likely. Radioactive bile collected from one animal (donor), and 1 ml. was then injected into the duodenum of a second rat which was also cannulated for bile collection. Samples of bile from the recipient became radioactive in about 10 minutes, and this animal showed definite signs of analgesia. Apparently, radioactive methadone was excreted through the intestinal route and, in addition, portions were reabsorbed and distributed throughout the body in sufficient amounts to produce analgesia.

Respiration of human brain slices and the effects of morphine and methadone on oxygen uptake. H. W. ELLIOTT (by invitation), V. C. SUTHERLAND and E. B. BOLDREY. *Divisions of Pharmacology and Experimental Therapeutics and of Neurological Surgery, Univ. of California Medical School, San Francisco, Calif.* Samples of cerebral cortex were obtained from patients undergoing surgical removal of various types of brain tumors. The tissue was immediately iced, transferred to a cold box and prepared for respiration studies. Pieces of cortex were isolated with scissors, sliced freehand with a razor and template, weighed and placed in Warburg flasks. The data obtained from 7 human brains studied to date follow: The QO_2 of human cerebral cortex slices in glucose-Ringer-PO_4, 30 minutes after equilibration at 37°C., was approximately 1.0 on a wet weight

basis. One hundred fifty minutes later the values for oxygen uptake had fallen 20–30%. For purposes of comparison 30-minute QO_2's for dog and rat cortex were 1.7 and 2.3, respectively. These species differences are to be expected because of the inverse relationships between metabolic rate and body size. Human cortex slices exhibited a striking difference in behavior from rat cortex slices when O_2 uptake was determined in Ringer-PO_4 containing no glucose. Rat cortex QO_2's fall very rapidly in the absence of substrate but human cortex respiration is not affected. QO_2's were just as high and as well maintained over a 3-hour period in unfortified Ringer-PO_4 as when glucose was added. The nature of the substrate apparently stored in human cortical tissue is as yet undetermined. To some vessels morphine and methadone were added after a 90-minute control period. The drug levels studied were *1*) 0.002M morphine HCl, *2*) 0.0005M methadone HCl and *3*) 0.002M methadone HCl. The O_2 uptake of both rat and human cerebral cortex slices in glucose-Ringer-PO_4 was not affected by *1*), stimulated by *2*) and strongly inhibited by *3*). The respiration of human brain slices in Ringer-PO_4 without added glucose was not affected by *1*) and inhibited by *2*). A comparison of the findings on rat and human brain indicates that the effects of 0.0005M methadone are on glucose utilization and may be a manifestation of enzyme inhibition rather than stimulation.

Metabolism of isopropyl alcohol by the perfused liver. Fred W. Ellis, *Department of Pharmacology, Univ. of North Carolina, Chapel Hill, N. C.* Rabbits, fasted for 48 hours, were anesthetized with 40 mg/kg. of pentobarbital sodium, intravenously, and prepared for liver perfusion by a modification of the technic of Lundsgaard, Nielsen and Orskov (*Skandinav. Arch. f. Physiol.* 73: 296, 1936). Fresh defibrinated, oxygenated rabbit blood diluted to 70 per cent with 0.9 per cent saline was perfused through the *in situ* liver, maintained at body temperature, at the rate of 72 cc/min. for a duration of 2 hours. At certain time intervals, the concentrations of sugar, acetone and lactic acid (and alcohol, when present) in the perfusion fluid were determined and liver samples were obtained for glycogen analyses. When isopropyl alcohol was added to the perfusion fluid, there occurred a distinct increase in acetone (a known oxidation product of isopropyl alcohol), as well as a less marked rise in sugar concentration. There was a corresponding decrease in the concentration of isopropyl alcohol. Changes in lactic acid and glycogen values did not seem to be significantly different from the control data. Upon the substitution of ethyl alcohol in the perfusion fluid there was no increase in acetone or sugar, and the rate of disappearance of the alcohol appeared to be somewhat slower than in the case of isopropyl alcohol. Lactic acid concentration remained practically constant as compared with the progressive decrease in the control and isopropyl alcohol experiments. There was no significant change in liver glycogen.

Photoelectric estimation of l-carnosine and l-histidine. G. A. Emerson and Paul L. Ewing. *Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas.* The reaction of Pauly (*Ztschr. f physiol. Chem.* 42: 508–18, 1904) may be adapted to photoelectric colorimetry of carnosine and histidine with certain modifications of the visual method of Koessler and Hanke (*J. Biol. Chem.* 39: 497–519, 1919) proposed by Kuen, Eggleton and others. A Coleman model 11 spectrophotometer was used, at λ 470 mu. Molar color values of carnosine and carbobenzoxycarnosine are higher than that of histidine if estimated immediately after diazotization, but approach that of histidine in 30 and 60 minutes, respectively, after which fading is negligible at a room temperature of 32° C., although the apparent spectral transmittance curves still differ somewhat for the secondary minima. Excellent agreement with the Lambert-Beer law obtains with 20–100 α of carnosine or histidine, and the method is suitable for estimations with neutralized Cl_3CCOOH extracts of tissues.

A comparative study of anticonvulsant activities of antiepileptic drugs by electro-shock methods in cats, rats and mice. Charles R. Ensor (by invitation) and Graham Chen. *Research Laboratories, Parke, Davis and Company, Detroit, Mich.* The anticonvulsant activities of nine antiepileptic drugs were determined with 2 electro-shock procedures, *1*) by the increase of electro-convulsive threshold in the cat (Putnam and Merritt) and *2*) by the abolition of extensor tonic seizures in rats and mice (Toman, Swinyard and Goodman). The values obtained with the 2 methods agree well in the cat and the mouse in the following order of potency: Dilantin, Mesantoin, Nirvanol, phenobarbital, Mebaral, Phenurone, Carbromal, Epidon and Tridione. In the rat, on the other hand, the protective doses of Mesantoin and phenobarbital were found to be smaller than those of others. The laboratory data are compared with the clinical results for these drugs in the treatment of grand mal epilepsy. They appear to show a fair agreement.

Pharmacological studies of phenacetylurea (Phenurone), an anticonvulsant drug. Guy M. Everett (introduced by R. K. Richards). *Dept. of Pharmacology, Abbott Research Laboratories, North Chicago, Ill.* Phenacetylurea (Phenurone) has outstanding anticonvulsant properties against Metrazol and supramaximal shock seizures in mice, rabbits and cats with non-sedative doses (300 mg/kg.). The drug also raised convulsive threshold to minimal electroshock seizures in rabbits. The toxicity is low, the LD_{50} by IP or oral administration being 2

to 3 gm/kg. Slight ataxia appears at 400 mg/kg. The EEG in unanesthetized cats and rabbits remained unchanged with doses up to 1 gm/kg. Higher doses produce high voltage slow wave activity. The duration of anticonvulsant action in mice is 3 to 5 hours. The duration remained unchanged in double nephrectomized mice, but was significantly prolonged (20 hours) by CCl_4 liver damage and hepatectomy. In man Phenurone causes signs of stimulation more often than sedation. The drug has been found effective in some resistant grand and petit mal cases and is particularly useful in psychomotor epilepsy.

Comparative effects of adenosine, isoguanosine and related compounds on smooth muscle. PAUL L. EWING, FRITZ SCHLENK and G. A. EMERSON. *Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas, and Dept. of Bacteriology, Iowa State College, Ames, Iowa.* Isoguanosine (2-oxyadenosine) was found to be about 3 times as active as adenosine in depressing the anesthetized rabbit's blood pressure after intravenous injection, in inhibiting rhythmic contractions of isolated rabbit or hamster intestine, and in stimulating the hamster uterus. On the isolated guinea pig uterus, isoguanosine was 60–100 times as effective a stimulant as adenosine. The related pyrimidines, cytosine and isocytosine, while much less active than adenosine, show the expected biochemorphic relationships. Although *in vitro* studies have demonstrated the production of glutamine and inosinic acid from ATP, glutamate and ammonia with tissue enzymes, we were unable to find any gross evidence of the reverse process, i. e., the production of active amounts of adenylic acid from a combination tion of inosinic acid and either glutamine or asparagine as amino donors.

Action of some enzyme inhibitors on the activity of the isolated intestine. A. FARAH, R. ANGEL (by invitation) and T. C. WEST (by invitation). *Dept. of Pharmacology, School of Medicine, Univ. of Washington, Seattle, Wash.* Isolated intestinal strips of the rabbit were placed in glucose-free Tyrode solution. After 30–60 minutes the intestinal strip showed a marked reduction in the height of spontaneous contractions and usually a loss in tone. In confirmation of results of previous workers, the addition of glucose, mannose, acetate, pyruvate and a number of fatty acids produced a recovery of the spontaneous contractions. The enzyme inhibitors malonic acid, p-chloromercury benzoate, sodium fluoroacetate, dinitrophenol, propionic acid, sodium cyanide and sodium azide were studied. The effect of some of these inhibitors was dependent on the type of substrate employed. The results will be presented and discussed in the light of our knowledge of the intermediary metabolism of carbohydrates and fatty acids.

Method for determining hydroquinone excretion in the urine. DAVID W. FASSETT. *Laboratory of Industrial Medicine, Eastman Kodak Company, Rochester, N. Y.* Because of the current interest in hydroquinone as an antioxidant for use in foods, and in the possible use of sodium gentisate in rheumatic fever, a study has been made of the excretion of hydroquinone in the urine of man and animals. The lack of specificity and sensitivity of available methods has been overcome by a modification of the procedure described by Sterner, Oglesby and Anderson for quinone vapors. (*J. Indust. Hyg. & Toxicol.* 29: 60, 1947.) The intense color formed by the simple addition of hydroquinone to an alkaline phloroglucinol solution has a characteristic absorption spectrum with two maxima at 420 and at 515 millimicrons. Catechol gives an equally intense but entirely different color, and resorcinol and phenol give no color under the same circumstances. Conjugated hydroquinone is estimated after hydrolysis of the urine with normal sulfuric acid. Amount of less than one microgram can be detected. Hydroquinone in both free and conjugated form (mostly the latter) is normally present in the urine of men, rats, guinea pigs, and cats. About 20–50 mg/24 hours is estimated to be a normal value for man, although this will vary with the type of diet. After ingestion of hydroquinone by mouth or by injection, large amounts are excreted in the conjugated form starting in an hour or two, indicating that the body eliminates hydroquinone rapidly. Compounds such as nor-dihydroguaiaretic acid also give a characteristic color with alkaline phloroglucinol but are less rapidly excreted.

Coenzyme I—Quinine relationships in bacterial growth and metabolism. R. M. FEATHERSTONE and J. R. PORTER (by invitation). *Depts. of Pharmacology and Bacteriology, College of Medicine, State Univ. of Iowa, Iowa City, Iowa.* It has been reported by others that coenzyme I will reverse the inhibitory effects which quinine has on the metabolism of *Escherichia coli*. In the work to be reported, turbidimetric measurements of growth, methylene blue reduction studies, experiments using standard manometric techniques, and microbiological assays for coenzyme I have been utilized in an investigation of quinine-coenzyme I relationships as they pertain to the growth and metabolism of several bacterial species. The results indicate that the apparent coenzyme reversal of quinine inhibition of methylene blue reduction by bacterial cells may be due to an oxidation of the coenzyme molecule. This oxidation is not inhibited by the concentrations of quinine used and it proceeds independently of the retarded glucose oxidation. It has also been shown that numerous other growth factors fail to reverse the quinine inhibition of growth or respiration of the several bacterial species studied.

Local anesthetic activity of a series of amino-alkoxy isoquinolines. EDWIN J. FELLOWS and EDWARD MACKO (by invitation). *Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Penna.* The following compounds were tested for local anesthetic activity after topical application to rabbits' eyes: 1-(beta-Dimethylaminoethoxy)-isoquinoline Monohydrochloride (I); 1 - (beta - dimethylaminoethoxy) - 3 - propylisoquinoline Monohydrochloride (II); 1-(beta-Dimethylaminoethoxy)-3-butylisoquinoline Monohydrochloride (III); 1-(beta-Diethylaminoethoxy)-3-methylisoquinoline Monohydrochloride (IV); 1-(beta-Di-n-butylaminoethoxy)-isoquinoline Monohydrochloride (V); 1-(beta-N-piperidylethoxy)-3-methylisoquinoline Monohydrochloride (VI); 1 - (beta - N - piperidylethoxy - 3 - ethylisoquinoline Monohydrochloride (VII); 1-(3-Diethylaminopropoxy)-3-ethylisoquinoline Monohydrochloride (VIII); 1 - (beta - Benzylaminoethoxy) - 3 - ethylisoquinoline Monohydrochloride (IX). All of the present derivatives exhibited anesthetic activity. Derivatives nos. II, IV, VI, VII, VIII and IX produced anesthesia for periods of 26 to 94 minutes in 0.01% concentration. However, no. III was outstanding in that an average of 75 minutes anesthesia was noted after application of 0.001% solution. In comparative studies anesthesia was noted for a period of 18 minutes after 1.0% cocaine and for an average duration of 69 minutes after 0.01% dibucaine hydrochloride.

Influences of progesterone and testosterone on reactivity of rabbit myometrium to oxytocin and vasopressin. PAUL P. C. FENG (by invitation) and R. A. WOODBURY. *Division of Pharmacology, Univ. of Tennessee, Memphis, Tenn.* Investigations were conducted on isolated uterine strips from rabbits, varying in age from 3 months to 3 years, with or without previous pregnancies. Twenty-one rabbits were sensitized by injecting 0.01–0.02 mg. of stilbestrol daily for a period of 7 days. (Control studies showed that such treatment produced full sensitization of the myometrium to oxytocin and vasopressin.) After sensitization 1 uterine horn was removed to test its reactivity to oxytocin and vasopressin. These results were then compared with the reactivity of the remaining horn removed after 5 days additional treatment with various combinations of stilbestrol and either progesterone or testosterone. In 8 rabbits, a stilbestrol/progesterone ratio of 1:20 to 1:100 decreased the reactivity of sensitized myometrium to oxytocin and vasopressin to similar degrees varying from five-fold to one hundred and fifty-fold. In 13 rabbits, a stilbestrol/testosterone ratio of 1:100 to 1:400 decreased the reactivity to oxytocin and vaspressin from 2-fold to 330-fold. However, testosterone decreased to a greater extent the reactivity to vasopressin in 9 of the 13 rabbits and to the same extent in 3 of the remaining 4. In stilbestrol-sensitized and non-sensitized rabbits, the oxytocic activity of vasopressin was roughly 20 times that as specified in the current N.N.R. These results are comparable with those reported by Bachinski and Allmark on normal rabbits (*J. Am. Pharmacol. Assoc.* 34:73, 1947).

Action of 4-amino, N 10-methyl, pteroylglutamic acid in mice, rats, and dogs. F. C. FERGUSON, JR. (by invitation), J. B. THIERSCH (by invitation), and F. S. PHILIPS. *Dept. of Pharmacology, Cornell Univ. Medical College, and Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.* The intraperitoneal LD_{50} of 4-amino, N 10-methyl, pteroylglutamic acid in mice was 94 ± 8.8 mg/kg in a single dose and 1.94 ± 0.30 mg/kg/day given for 5 successive days; and in rats, 12.6 mg/kg acutely (with much variation) and 1.11 ± 0.34 mg/kg/day for 5 successive days. Orally in rats the acute LD_{50} was 180 ± 45 mg/kg. Signs of intoxication included weight loss, anorexia, diarrhea, dehydration, and weakness, complicated by secondary infections, with death after 3 days. Rats sacrificed within 72 hours after large acute doses and after chronic doses given for 60 days, showed lesions in the bone marrow and intestines, the lymphoid tissues remaining relatively unaffected. The intestinal mucosa showed hyperemia, edema, desquamation and leucocytic infiltration. The marrow became fluid and hypocellular in the myeloid and erythroid elements. The blood showed progressive normochromic normocytic anemia, reticulocytopenia, and leucopenia. Dogs receiving various doses were followed with serial blood counts and sternal marrow aspirations. The course of intoxication and the lesions seen were similar to those in rats. In addition, megaloblasts appeared in the marrow, the lymphoid tissues appeared reduced and ulcerative colitis was prominent.

Comparison of the chronic toxicity of alpha, beta and gamma isomers of benzene hexachloride. O. G. FITZHUGH, A. A. NELSON and OMA L. HOLLAND (by invitation). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Following our observation that technical grade benzene hexachloride was more toxic than could be accounted for by the gamma content, a chronic experiment on the individual isomers was started. Albino rats, 21 days old, 20 to a group, were fed dosages of 10, 100 and 800 ppm of the alpha, beta and gamma isomers in a stock diet for their lifetime. Additional dosage levels of 5, 50 and 1600 ppm gamma and 50 ppm alpha were used. Results showed that the beta isomer was more than twice as toxic as the gamma and that the alpha isomer was of the same order of toxicity as the gamma. All rats receiving the 800 ppm beta isomer died within the first 10 weeks of

the experiment, whereas some of those on 1600 ppm gamma survived for more than a year. Retardation in growth occurred at the 800 ppm dosage level for each isomer. Lower dosage levels did not affect growth. Rats on the high dosages of the gamma isomer had severe convulsions. At 100 ppm all isomers produced slight but distinct histological changes in the liver, and, with the alpha and gamma, in the kidney also. The beta affected the liver in a greater degree at a given level. The gamma and alpha isomers produced no damage to the liver or kidney at the 10 ppm and the beta at this level showed a questionable injury to the liver.

Mercury content of human tissues from routine autopsy material. ROBERT B. FORNEY (introduced by H. R. HULPIEU) and R. N. HARGER. *Dept. of Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis, Ind.* Using the method of Laug and Nelson (*J. Assoc. Off. Agr. Chem.* 25: 390, 1942) mercury analyses were run on kidneys and livers from 117 consecutive autopsies. These comprised: *group A*, 92 persons with no known mercury medication; and *group B*, 25 persons with a history of the administration of mercury diuretics. In *group A* the frequency distribution of mercury concentration expressed as mg. % of the fresh organ was, kidneys: 9, none (< 0.01 mg. %); 20, 0.01–0.10; 28, 0.11–0.50; 22, 0.51–1.00; and 11, 1.01–12.7; and liver: 18, none; 37, 0.01–0.10; 26, 0.11–0.50; 5, 0.51–1.00; and 3, 1.01–1.72. Concentrations of mercury above 0.1 mg. % were found in 67.5% of the kidneys and 34.5% of the livers. In *group B* the distribution of mercury levels was, kidney: 1, 0.94; 12, 1.1–5.0; 6, 5.1–10.0; 3, 12.0–15.0; 1, 21.7; and 1, 27.5; and liver: 15, 0.11–0.50; 4, 0.51–1.00; and 3, 1.1–2.5. In all of the cases in *group A* and in most of the cases in *group B* there was no microscopic evidence of the pathological changes associated with acute mercury poisoning, although a few of *group B* showed mild changes. In 24 of the cases separate analyses were run on the cortex and medulla of the kidney. In 22 of them the cortex/medulla ratios varied from 1.1 to 6.8 The highest level of mercury was found in the kidney with the liver next, followed by the spleen.

Respiration apparatus for rabbits and its use in the study of piperidine analgesics. R. H. K. FOSTER and B. DEBOER. *Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, Mo.* The apparatus, which eliminates the use of respiratory valves, consists of a closed two-compartment rabbit holder, a 40 gallon air reservoir with a blower for introducing fresh air, 2 damped float recorders and an air circulating fan. A perforated rubber diaphragm fitting snugly around the rabbit's neck separates the two compartments of the rabbit holder. The larger compartment, holding the rabbit's body, is connected to one float recorder for registering rate and depth of respiration; the smaller, holding the rabbit's head, is connected in turn to the soda lime container, the air reservoir, the small air circulating fan and thence back to the head compartment. An outlet from the air reservoir is connected through a fine capillary to the second float recorder for recording oxygen consumption. The damping effect of the capillary prevents oscillations due to breathing and results in practically a smooth line. Morphine sulfate and dl - α - 1,3 - dimethyl - 4,4 - phenylpropionoxypiperidine hydrochloride (Nu-1196) were injected in doses of 2 and 5 mg/kg. Both drugs produced about equally a marked decrease in rate and minute volume but tidal volume and oxygen consumption showed no significant changes from the control period. The onset was slightly faster with Nu-1196 and recovery definitely faster. Other members of the series are under investigation.

Effects of posterior pituitary hormones on carbohydrate metabolism. ALEXANDER MACL. FRASER. *Dept. of Pharmacology, McGill University, Montreal, Canada.* The hyperglycemic action of pituitary extract is well known; it has been attributed to the oxytocic hormone in the case of the dog. Hyperglycemia following administration of glucose or adrenaline decreases blood inorganic phosphate, due to increased insulin secretion, but workers have shown that pituitary extract increases blood inorganic phosphate, and that pitressin antagonizes the fall in inorganic phosphate caused by insulin. In the present work, the effects of highly purified preparations of the oxytocic and pressor hormones on the sugar and inorganic phosphate of the blood were studied in dogs. The results show a drop in phosphate following oxytocic hormone, and a rise following the pressor hormone. Blood sugar rose after injection of each hormone. The hyperglycemic action of the pressor preparation cannot be accounted for, quantitatively or qualitatively, by its contamination with oxytocic hormone; therefore a hyperglycemic action is attributed to the pressor hormone. It appears likely that the decrease in plasma phosphate after the oxytocic hormone is due to increased insulin secretion, which is caused, in turn, by the hyperglycemia. Consideration of the doses of the pressor hormone required to produce the above changes, leads to the suggestion that the increases in phosphate and sugar following administration of this hormone are due to tissue anoxia, which results from the vasoconstriction; anoxia is known to produce such changes. Hyperglycemic doses of the oxytocic hormone are within a more physiological range.

Rate of disappearance of isomers of benzene hexachloride from fat depots in rats. JOHN P. FRAWLEY (by invitation) and O. GARTH FITZHUGH. *Division of Pharmacology, Food and Drug*

Administration, Federal Security Agency, Washington, D. C. Previous experiments in this laboratory have shown that the chronic storage of the beta isomer of benzene hexachloride was considerably greater than that of the other isomers and that the principal storage took place in fat tissue. Data are presented here to show the various rates of disappearance of the isomers from the fat tissue. Rats were fed the isomers of benzene hexachloride at 100 ppm and 500 ppm until the maximum storage level was reached. Fat tissues from both sexes on the isomers were analyzed at the maximum level of storage, and repeatedly thereafter until the level fell below the sensitivity of the method of analysis. The method was based on the conversion of the benzene hexachloride to 1,2,4-trichlorobenzene and the estimation of this compound by means of the ultraviolet spectrophotometer. The results obtained from the female rats are summarized in the following table:

Disappearance of Isomers of B.H.C. in Female Rats

Concentration Original Diet	Weeks Control Diet	Conc. of Isomers (Mg/g.)			
		alpha	beta	gamma	delta
ppm					
100	0	0.177	1.014	0.102	0.128
	1	0.142	0.860	0	0
	2	0	0.837		
500	0	1.180	No	0.281	0.376
	1	0.281	survivals	0.039	0.188
	2	0.158		0	0

The disappearance of the isomers from fat tissue of male rats is accomplished sooner than in female rats.

Effects of indophenols on glycolysis. JAMES A. FREEK (introduced by G. L. GEMMILL). *Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville, Va.* A study was made of the effect of some indophenols on glycolysis in frog muscle extracts. The rate of glycolysis was determined by the liberation of carbon dioxide from a bicarbonate buffered solution in a Warburg vessel. The following compounds completely inhibited glycolysis at .0001 to .0008 M: 2,6-dibromo-3′-carboxyindophenol, 2,6 - dichloro - 3′ - chloroindophenol, 2,6-dibromoindophenol and 2,6-dichloro-3′-methylindophenol. The following compounds partially inhibited at .0006 to .003 M.: 2,6-dichloroindophenol, 2,6 - dibromo - 3′ - methoxyindophenol, 2,6 - dibromo - 2′ - methyl - 5′ - isopropylindophenol, 3′-methyl-6′-isopropylindophenol, 2′-methyl-5′-isopropylindophenol, 3′-methylindophenol, 2′-methylindophenol, 2,6 - dibromo - 3′ - methylnol, 2′ - methylindophenol, 2,6 - dibromo-3′ - methyl - indophenol, indophenol, and 3′-naphtholsufonate. 2,6-Dichloroindophenol, 2,6-dibromo-3′-carboxyindophenol and 3-chloroindophenol at .00001 M. increased the amount of carbon dioxide production. This activiting effect was dependent on the normal activity and reducing capacity of the extract. The inhibition produced by 2,6-dibromo-3′-carboxyindophenol and 3′-naphtholsulfonate was not reversed by glutatione or cysteine. Inhibition by 2,6-dibromo-3′-carboxyindophenol was reversed in the presence of added ATP. There was no quantitative relationship between inhibition and the oxidation—reduction potentials of the indophenols.

Responses to epinephrine and norepinephrine after dibenamine. WALTER A. FREYBURGER (by invitation), LUIS R. CAPO (by invitation), and GORDON K. MOE. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* In the intact dog dibenamine 'reverses' the pressor action of epinephrine in doses which depress but do not abolish or reverse the pressor action of norepinephrine. 'Reversal' of epinephrine is thought to represent unmasking of its vasodilator activity. Since a a depressor action of epinephrine is rarely apparent in the pithed animal, one might expect that the pressor effects of the two amines would be depressed to an equal degree by dibenamine in this preparation. This was found to be the case. In a series of intact anesthetized dogs dibenamine, in a dose which reduced the maximum response to epinephrine to a pressure rise of 5 mm. Hg followed by a fall, reduced the pressor action of norepinephrine by only 50%. In the pithed cat, pressor responses to the two amines were reduced to exactly the same degree by dibenamine. If the vasodilator action of epinephrine depends upon an intact autonomic nerve supply, interruption of such pathways by tetraethylammonium (T.E.A.) should abolish vasodilator responses. T.E.A. was found to abolish the pressure fall normally observed after small doses of epinephrine. The depressor response to epinephrine which occurs following dibenamine, however, was only occasionally prevented by T.E.A. Although the difference may be only quantitative, there is a possibility that the vasodilator effect of epinephrine following a reversing agent differs from the normal vasodilator effect demonstrable with small doses.

Paper chromatography of flavonoid pigments. II. Separation and quantitative estimation of rutin and quercetin. THOMAS B. GAGE and SIMON H. WENDER (introduced by ARTHUR A. HELLBAUM). *Chemistry Dept., Univ. of Oklahoma, Norman, Okla.* The qualitative separation and identification of micro quantities of 11 flavonoid pigments by paper partition chromatography has been previously reported by the authors (*Science*. In press). A micro method of quantitatively estimating the amounts of certain flavonoid pigments initially present in a mixture has now been developed. Mixtures of rutin and quercetin have been separated and quantitatively determined

by the following procedure. Whatman No. 1 filter paper strips, 2.5 × 50 cm. were spotted by means of a micro pipette with 5–10 μl. of a solution containing 10–40 μg. of each pigment. The strips were chromatographed in a one-dimensional apparatus with such solvent combinations as ethyl acetate-water or n-butanol-acetic acid-water (40-10-50 vol.%). Blank strips were treated similarly in order to provide solvent blanks for the preparation of absorption spectra curves. The strips were air-dried, and the pigment zones located by their fluorescence in ultraviolet light. The isolated pigment zones and the corresponding areas of the blank strip were cut out and the paper extracted in closed containers with solvents such as 5 cc. of ethanol containing 0.02 cc. of concentrated HCl. The Beckman model DU quartz spectrophotometer, fitted with micro absorption cells, was used for obtaining spectral data. Rutin and quercetin were identified by the shape of their characteristic ultraviolet absorption spectra curves and the amount recovered was calculated from their extinction values at 362.5 and 375 mμ. respectively. Further studies are in progress with combinations of 2 and 3 flavonoid pigments often found associated together in natural products.

Studies on furan compounds: toxicity and pharmacological action of furfuryl alcohols. JOHN E. GAJEWSKI and WILLIAM R. ALSDORF (introduced by C. H. HINE). *Naval Medical Research Institute, Bethesda, Md.* Upon oral administration of a 2.0% aqueous solution of furfuryl alcohol, the LD-50 for white rats is 275 mgm per kilo. Administered intravenously as a 10% aqueous solution to rabbits, the LD-50 is 650 mgm per kilo. The marked difference between oral and intravenous toxicity can probably be explained on the basis of destruction of furfuryl alcohol by the gastric juice, since this substance is very unstable in an acid medium. This difference does not exist with the more stable hydrogenated analog, tetrahydro-furfuryl alcohol, (oral LD-50 for white rats, 4500 mgm per kilo; intravenous LD-50 for rabbits, 725 mgm per kilo). Either alcohol chronically administered to rats in the drinking water over a period of 20 days results in marked anorexia and weight loss. Intravenous injection of either alcohol into rabbits produces a flaccid paralysis, which becomes permanent with large doses. Recovery from smaller doses is complete in a period of about thirty minutes. On smooth muscle furfuryl alcohol exerts a slight antispasmodic effect. In dogs, upon intravenous injection, the depression of respiration is not accompanied by any changes in blood pressure until doses near the lethal are given. In all species death results from respiratory paralysis.

Metabolism of radioactive nicotine. A. GANZ (introduced by F. E. KELSEY). *Dept. of Pharmacology, University of Chicago, Chicago, Ill.* The biosynthesis of C^{14} labeled radioactive nicotine has been reported by Geiling, Kelsey, McIntosh and Ganz in *Science* 108: 558, 1948. Using radioactive nicotine with a specific activity of 57,000/min/mg., rats and mice were injected intravenously and the distribution of the drug among the various organs and body fluids was studied. Distribution and fixation studies on the isolated heart of the guinea pig were also carried out with simultaneous analyses being made of the perfusion fluid. The relationships between the tissue data, the perfusate analyses and the kymograph records were investigated.

Effects of naphthoquinones and related compounds on glycolysis. CHALMERS L. GEMMILL. *Dept. of Pharmacology, University of Virginia Medical School, Charlottesville, Va.* The study of the action of some of the naphthoquinones and related compounds on muscle glycolysis reported last year (*Federation Proc.* 7: 220, 1948) has been extended. A detailed investigation was made of the effects of various concentrations of sodium 1,2-naphthoquinone-4-sulfonate on glycolysis of glycogen to lactic acid in muscle extracts of frogs. With small amounts (1×10^{-4} Molar) of this compound a slight stimulation of glycolysis was observed, while larger amounts caused a progressive decrease in glycolysis, with practically complete inhibition occuring at concentrations of 6×10^{-4} Molar. Several of the compounds having Vitamin K activity gave partial inhibition of glycolysis in amounts of 0.002 Molar or less. These compounds were 2-methyl-1,4-naphthoquinone (Eastman), sodium - 2 - methyl - 1,4 - naphthohydroquinone diphosphate (Parke Davis Co.) and 2-methyl-4-amino-1-naphthol hydrochloride. 2-methyl-3-hydroxy-1,4-naphthoquinone (Phthiocol) gave partial inhibition of glycolysis in a concentration of 0.001 Molar. A polarographic examination (carried out by Mr. J. G. Hilton) of the sodium 1,2-naphthoquinone-4-sulfonate in the muscle enzyme solution revealed that from 80 to 90% of this compound is destroyed or bound in its reaction with the glycolytic mechanisms.

Chemotherapeutic efficacy of nitrofurazone (furacin) in experimental trypanosomiasis of the mouse. N. J. GIARMAN (by invitation), B. A. RUBIN and E. L. MCCAWLEY. *Laboratories of Pharmacology and Bacteriology, Yale Univ. School of Medicine, New Haven, Conn.* Single oral or subcutaneous doses of 500 mg. of nitrofurazone per kg. of body weight were capable of curing mice which had been given a massive, lethal infection of *Trypanosoma equiperdum* (one million organisms injected intra-abdominally). There were no therapeutic failures at this dose level in 120 mice, some of which were observed for 180 days after infection. The average survival time of infected but un-

treated animals was 84 hours. The chemotherapeutic index for nitrofurazone, calculated on the basis of LD-5/ED-95 × 100, is 107 when the drug is administered orally, and 117 when it is given subcutaneously. These indices compare favorably with those of such trypanocidal antimonials as fuadin and stibenyl, which were calculated in the same manner from the data of Chen *et al.* (*J. Infect. Dis.* 76: 144, 1945.) on intramuscular therapy of trypanosomiasis, as 87 and 143 respectively. When the infecting inoculum was increased to 10 million organisms, the dose of 500 mg/kg. was still capable of curing 100% of the experimental animals. When treatment was withheld for 60 hours, at which time the first inoculated control animal had succumbed to the infection, the dose of 500 mg/kg. subcutaneously cured 50% of the animals. Nitrofurazone has a low order of toxicity. Doses of the drug in the magnitude of 30 to 50 mg/kg. given intravenously to nembutalized dogs and cats failed to produce any profound toxic changes in the blood pressure, EKG, EEG, or respiration. The LD-50 for nitrofurazone administered orally to the mouse is 737 mg/kg., leaving a convenient margin of safety from the effective dose of 500 mg/kg.

Pharmacological studies on actidione. ANDRES GOTH and FABIAN J. ROBINSON (by invitation). *Dept. of Physiology and Pharmacology, Southwestern Medical College, Dallas, Texas.* The antibiotic actidione has been shown to be markedly inhibitory for certain pathogenic fungi (Whiffen, A. J., *J. Bact.*, 56: 283). It also showed a marked species variation in its toxicity for mammals. Despite its toxicity it seemed of interest to study blood levels of the antibiotic in several species of animals. A solution of crystalline actidione was injected intraperitoneally into rats, dogs, and guinea pigs. The citrated plasma of these animals was tested by a serial dilution method in tryptose phosphate broth. The test organism was *Saccharomyces pastorianus*, ATCC 2366, and the inoculum was adjusted to give a final concentration of 1:250 of a 24 hour culture. In the rat the injection of 1 to 10 mg/kg. of actidione was followed in 30 minutes by plasma levels of 5–20 dilution units per cc. The inhibitory activity decreased in 90 minutes but was still demonstrable. In the dog and the guinea pig the results were essentially the same as in the rat. These data indicate that actidione is rapidly absorbed from the intraperitoneal site in the rat, dog, and guinea pig and is demonstrable in plasma for at least 90 minutes. Since inhibitory blood levels can be obtained following the injection of sublethal doses of actidione, the possibility exists that the compound may exert a chemotherapeutic effect against susceptible pathogenic fungi in experimental animals and man.

Modified ultraviolet spectrophotometric method for quantitative determination of barbiturates. THELMA C. GOULD, ELIZABETH L. MAYO, and KARL P. BOWMAN (introduced by CHARLES H. HINE). *Divisions of Pharmacology and Experimental Therapeutics and of Psychiatry, Univ. of California Medical School and Langley Porter Clinic, State Dept. of Mental Hygiene, San Francisco, Calif.* Diethyl ether rather than chloroform was used to remove the barbiturate from the sample of blood. A semi-micro, continuous extractor permits direct extraction of from 0.5–5 ml. sera. This procedure is less time-consuming than previous methods and has simplifications regarding apparatus, procedure, and glassware. By extraction at the normal *pH* range of serum, all sulfonamides thus far tested, which may contribute to ultraviolet absorption, are eliminated. The major problem arising in spectrophotometric determination of barbiturates is the ultraviolet absorption by other extractable materials present in blood sera. In experimental work, the optical density of sera can be determined prior to administration of barbituarate, and correction made. In forensic toxicology, however, where no blank specimen is available, this is not possible. To account for this possible error, the optical density of normal human sera was determined in 100 samples. This variation was from 0.040 to more than 0.200 at a wave length of 245 mu., with the majority of sera falling within the range of optical density of 0.100–0.150, with a mean of 0.130. When this value is used as a blank reading in barbiturate-containing serum, the error involved would probably not exceed 0.5 mg.%. The *pH* should be carefully adjusted between 9.5 and 10.0 when reading optical density, to detect the typical lactam-barbiturate curve. This is not obtained otherwise, and its maximum may be obscured by the serum blank.

Synergistic chemotherapeutic effect of penicillin and aurothioglucose (Solganal). ARNOLD GRANDAGE, LEILA DIAMOND, MARVIN ROSENKRANTZ, JEANNE H. CHASE, RICHARD TISLOW and ERWIN SCHWENK (introduced by H. B. Haag). *Biological Research Labys., Schering Corporation, Bloomfield, N. J.* The chemotherapeutic effects of aurothioglucose (Solganal) and penicillin were studied in albino mice infected with *Borrelia novyi* (culture obtained through the courtesy of Dr. R. J. Schnitzer, Hoffmann-La Roche, Nutley, New Jersey). A shortened assay procedure similar to that described by Buck, Farr and Schnitzer (Science 104: 307, 1946) was used. Mice inoculated intraperitoneally with standardized dilutions of blood in Tyrode solution from mice carrying infection of *Borrelia novyi* were treated in separate experiments with graded doses of penicillin or aurothioglucose and checked for clearance of the blood from *Borrelia novyi* at the end of 24 hours. A satisfactory dose response relationship was obtained with both drugs. Also a satisfactory dose

response relationship between graded inocula and clearance was found using 1 dose of aurothioglucose or penicillin. On the basis of these preliminary experiments 4 short-term experiments, each with groups of 6 animals per dose level, were run to determine the effect of combined treatment with aurothioglucose and penicillin. Aurothioglucose was injected intraperitoneally 4 hours before and crystalline sodium penicillin G immediately after infection. The results of each of the 4 experiments revealed a synergism of the 2 drugs. According to the combined data 8 U/gm. of penicillin cleared 37% and 0.05 mg/gm. of aurothioglucose 9% of the mice, whereas a combined treatment with the 2 drugs at these dose levels resulted in a 75% clearance. Similarly 8 U/gm. penicillin and 0.09 mg/gm. aurothioglucose cleared 37% and 13% respectively and the 2 drugs in combination 93% of the animals. These data indicate that combined therapy in this dosage range produced more than an additive effect.

Toxicity of ozone under simulated industrial conditions. M. G. GRAY. *Labys. of Arthur D. Little, Inc., Cambridge, Mass.* The use of ozone as an 'air-purifier' in garages has directed attention to the limited data regarding its actual toxicity in concentrations of the order of 1 ppm. An experimental study is reported in which rats, mice and rabbits were exposed to a) ozone, b) to exhaust fumes from an internal combustion engine and c) to an atmosphere combining a) and b). This was done in a gas chamber designed to simulate conditions in a service garage. The duration of exposure was equivalent for the species used to more than 1 year of average working time for garage employees. Neither ozone (1 ppm.), motor exhaust fumes (containing 50 ppm. carbon monoxide) or a combination of these produced significant changes in the physical condition, nutritional status or gross findings at autopsy. No evidence was obtained to support claims that ozone decreases the toxicity of carbon monoxide. The study was carried out in a gas chamber designed to permit the blending of several gases with normal atmospheres while maintaining constant physical conditions. By use of interchangeable panels, the size of the chamber can be altered to suit the requirements of several kinds of experimental work.

Xanthine studies: 1) effects of aminophyllin, 1, 3 diethyl 8 bromoxanthine and 1, 3 dimethyl 8 chloroxanthine on cardiac output. D. M. GREEN, W. C. BRIDGES, A. D. JOHNSON, J. H. LEHMANN, F. GRAY, and L. FIELD (all by invitation). *School of Medicine, Univ. of Washington, Seattle, Wash.* 1,3-diethyl-8-bromoxanthine and 1,3-dimethyl-8-chloroxanthine have been compared with aminophyllin as to effect on cardiac output. The subjects consisted of 45 patients with myocardial insufficiency. Output was measured by the Fick method. Total of output determinations numbered 236, of which 118 followed intravenous administration of a test drug. Aminophyllin was the most active compound and produced an average increase in output of 12% in doses of 250 mg. 1,3-diethyl-8-bromoxanthine was qualitatively similar to aminophyllin but the effect on output was approximately ½ as intense. 1,3-dimethyl-8-chloroxanthine was followed by a small net decrease. The actual effect on output was probably greater than indicated by comparison of post-injection with pre-injection values, for a fall in output of approximately 10% was observed when repeated determinations were made in close succession on controls. None of the 3 compounds uniformly affected cardiac output in a single direction. The percentage change following injection varied from plus 185 to minus 45%. On the average, patients with high pre-injection outputs manifested a fall in output following administration of the test drug, while patients with low pre-injection outputs showed a rise. Many individual variations were observed, however. Output changes were due largely to altered stroke volume. Increased output was associated with a greater fall in intra-atrial pressure, a decreased arteriovenous oxygen difference and a rise in systemic blood pressure.

Xanthine studies: 2) effects of aminophyllin, 1,3 diethyl 8 bromoxanthine and 1,3 dimethyl 8 chloroxanthine on water and sodium excretion. D. M. GREEN, W. C. BRIDGES, A. D. JOHNSON, J. H. LEHMANN, F. GRAY, and L. FIELD (all by invitation). *School of Medicine, Univ. of Washington, Seattle, Wash.* The renal actions of 1,3-diethyl-8-bromoxanthine and 1,-3-dimethyl-8-chloroxanthine were first observed by Newman and associates. In the present study these compounds have been compared with aminophyllin as to locus and intensity of effect. The subjects consisted of 25 hospital patients. Basal levels of renal function were measured during 2 20-minute clearance periods prior to intravenous injection of the drug. Drug effects were estimated by comparing control values with those obtained during 2 20-minute clearance periods following administration. All 3 compounds increased sodium and water excretion. Clearance data indicated these changes to be due almost entirely to a fall in percentage of tubular reabsorption. Aminophyllin was the most active of the compounds and doubled sodium output in 250 mg. doses. The rise in water excretion was less than half as great. The glomerular filtration rate averaged an increase of approximately 15%. 1,3-diethyl-8-bromoxanthine was ⅛–¼ as active as aminophyllin in augmenting sodium output. The effect on water excretion was proportionately less. Filtration rate changes were small and variable in direction. The renal effects of 1,3-dimethyl-8-chloroxanthine were inconstant and so weak as to

indicate the compound to be nearly inert. Analysis of aminophyllin action in relation to pre-injection levels of renal function showed the intensity of diuretic effect to be proportional to basal glomerular filtration rate. This relationship suggests that the effectiveness of the xanthines as diuretics depends on the renal functional reserve of the patient.

In vitro oxidation of oxophenarsine hydrochloride. RAY E. GREEN (introduced by A. L. Tatum). *Dept. of Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* The breakdown of oxophenarsine hydrochloride (mapharsen) on oxidation in air and 100% oxygen has been studied on various samples of the compound. The changes in LD-50 and minimal curative dose (M.C.D.) during the oxidation were determined for the albino rat and for *T. equiperdum* infection in the same species. An increase in the LD-50 and the M.C.D. has been noted on oxidation for both the aqueous solutions and the powdered compounds. When an aqueous solution is oxidized in pure oxygen for 24 hours or longer, a two- to three-fold increase in LD-50 (from 16 to 45 mg/kg.) results and a dose 2½ times the M.C.D. of the unoxidized compound is ineffective. The oxygen uptake in pure oxygen at 37°C has been followed manometrically and a linear relationship between oxygen consumption and time exists up to 25 hours of oxidation. Approximately 90% or more of the total decrease in toxicity occurs during this period. The effects of variations in *p*H on the rate of oxidation have been observed qualitatively over the range of *p*H 2.5 to 7. Increasing the *p*H value of the solution increases the rate of oxidation. At *p*H 2.5 and 25°C after 95 hours aeration, no demonstrable change in toxicity or therapeutic effectiveness was noted. On the other hand, at *p*H 7, for example, oxidation occurred very rapidly. In all instances examined, mapharsen upon oxidation became less toxic and correspondingly less effective as a trypanocide.

Effect of cholinesterase inhibitors on the permeability of dog erythrocytes in vitro. MARGARET E. GREIG and WILLIAM C. HOLLAND (by invitation). *Dept. of Pharmacology, Vanderbilt Univ. Medical School, Nashville, Tenn.* In this laboratory it has been found that the intravenous administration of methadon to dogs frequently was followed by an increase in erythrocyte fragility and occasionally by a hemoglobinuria. Methadon also produced an increased hemolysis of dog and human erythrocytes *in vitro*. In metabolic experiments *in vitro* we have found that methadon inhibited the glycolysis of glucose, and the oxidation of lactate, pyruvate and succinate by brain. (Arch. Biochem. 17, 129; 19, 441, 1948.) Methadon also inhibited cholinesterase activity. Wilbrandt (Pflüger's Arch. f. d. ges. Physiol. 243: 519, 1940) reported that sodium fluoride and sodium iodoacetate produced changes in permeability of erythrocytes which he attributed to their being glycolytic inhibitors. These compounds are, however, also inhibitors of cholinesterase activity. We have found that in addition to methadon certain more specific cholinesterase inhibitors which are reputed to have no effect on glycolysis, e.g., physostigmine, under certain conditions also produced changes in fragility of dog erythrocytes. The effect of these drugs was influenced markedly by the ionic composition of the medium.

New potent analgesic agent. E. G. GROSS, M. A. BROTMAN (by invitation), S. F. NAGYFY (by invitation), W. W. SAWTELLE (by invitation) and L. L. ZAGER (by invitation). *Depts. of Pharmacology, Anesthesiology, Obstetrics and Gynecology, Urology and Surgery. State Univ. of Iowa, Iowa City, Iowa.* A new analgetic agent, 3-hydroxy-N-methyl morphinan hydrobromide (Nu-2206, Hoffmann-LaRoche, Inc.) is being studied experimentally and clinically in the above named departments. In the department of pharmacology, the analgetic potency of Nu-2206 has been studied using the Wolff-Hardy technique with normal human subjects, and the compound appears to have approximately four times the analgetic potency of morphine sulfate. With the highest dose used, 3 mg. given subcutaneously, slight dizziness, nausea, vague gastro-intestinal symptoms and sedation were observed to a lesser degree than with a comparable analgetic dose of morphine sulfate. No euphoria was experienced by any of the subjects receiving the new agent. Blood pressure changes were slight and probably can be accounted for by the quieting of the subject. Respiration was only slightly depressed. Preliminary clinical reports indicate that the compound has about 3 times the potency of morphine sulfate when used in patients with incurable carcinoma and as a post-operative analgetic agent. The drug has also been used with moderate success in doses as high as 12 mg. intravenously as the sole preanesthetic agent for nitrous oxide anesthesia, and there is some indication that the analgesia persists for a greater duration than is usual with other analgesic compounds.

Effect of diethylaminoethyl ester of 1-phenyl-cyclo-pentane-1-carboxylic acid hydrochloride, 'parpanit', on decerebrate rigidity, spinal reflexes and skeletal muscle. CHARLES M. GRUBER, CHARLES P. KRAATZ (by invitation), CHARLES M. GRUBER, JR., and JOAN E. COPELAND (by invitation). *Dept. of Pharmacology, Jefferson Medical College, Philadelphia, Penna.* Seventeen decerebrated cats were used to study the effect of Diethylaminoethyl ester of phenyl-cyclopentane-carboxylic acid hydrochloride (parpanit) upon muscular rigidity. Four decapitated cats were used to determine the effect of parpanit upon reflexes, and 4 decerebrated cats and 11 urethanized dogs

to learn the effects of this drug upon motor nerve endings and on skeletal muscle, normal and denervated. Parpanit, trasentin and syntropan injected intravenously in adequate doses relieve the muscular rigidity of decerebrated cats. For this purpose these drugs were found superior to either atropine or scopolamine. The reflexes caused by mechanical stimulation of the back and paws in the spinal cat can be depressed by adequate doses of parpanit, trasentin and syntropan given intravenously. The height of the contractions of the anterior tibial muscle is increased with intravenous injections of parpanit. The intra-arterial injections of parpanit, trasentin and syntropan increase the height of the contractions of the anterior tibial muscle when their doses are small and rapidly injected and when their doses are large and slowly injected. Large doses rapidly injected cause a decrease in the activity of the muscle preceded and followed by increases above the height of the control. Parpanit, trasentin, syntropan, atropine and intocostrin cause dilatation of the blood vessels in skeletal muscle when injected intra-arterially. The increased and decreased muscular activity following injections of parpanit and trasentin are direct effects of these drugs on the muscle cells, independent of the increase in blood flow. The depression of decerebrate rigidity by parpanit is due to central action and not a curare-like action on the neuromuscular junction.

Ganglionic blocking action of 'Dibutoline'. Carl C. Gruhzit (by invitation) and Gordon K. Moe. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* Dibutoline, a quaternary ammonium compound with atropine-like properties, produces cardiovascular actions resembling those of tetraethylammonium. The ganglionic blocking action of Dibutoline was demonstrated in studies on arterial pressure, femoral arterial blood flow, and transmission through the stellate and superior cervical ganglia in dogs and cats. Intravenous injection of the drug causes a fall of pressure with an increased femoral blood flow, while intra-arterial injection causes little or no direct action on the vessels. Intravenous infusion blocks the respiratory and pressor actions of nicotine and high doses of acetylcholine and potentiates the pressor action of epinephrine. The effects of preganglionic stimulation of the cardiac sympathetic nerves on heart rate and of the cervical sympathetic trunk on the nictitating membrane are blocked by Dibutoline, while the effects of postganglionic stimulation are uninhibited. The doses necessary to produce ganglionic blockade greatly exceed those said to produce atropine-like effects and would be encountered only with the highest doses used clinically.

Relative local emetic activity of glycosides of the digitalis series in cats. Romulo Guevara (by invitation), Walter Modell, Theodore Greiner (by invitation), Melvin Moore (by invitation), Abraham Blumer (by invitation), Harold Evans (by invitation) and Harry Gold. *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* All glycosides of the digitalis series exert local irritant action in the gastrointestinal tract. Whether this action is equally well developed in different glycosides has never been satisfactorily examined. Four glycosides, ouabain, scillaren A, scilliroside, and lanatoside C were compared. These were selected because they were available in pure form, their molecular weights and structures are known, and they are poorly absorbed from the gastrointestinal tract. Each of a series of doses of each compound was administered by stomach tube to a group of cats on an empty stomach (in all over 250 cats), and the animals were placed under continuous observation for a period until the first sign of vomiting or for at least 12 hours. The percentage of animals in which there was an emetic response for each of the series of doses was plotted against the log of the dose. The results show that the property of local emetic activity is developed to widely different degrees in the molecular structure of the various digitalis glycosides, and the ones tested here show the following order of increasing approximate potencies: ouabain-1 : scillaren A-4 : lanatoside C-10 : scilliroside-10. That the emetic action was local was assumed from the fact that intravenous assay following emesis disclosed a systemic action of only from about 10 to 20% of the fatal dose. Local emetic action delayed in some cases for as long as 10 hours was an unexpected observation. There was no relation between the cardiac potency and the local emetic action.

Study of coca addiction. Carlos Gutierrez-Noriega. *Dept. of Pharmacology, Universidad Nacional Mayor de San Marcos, Lima, Peru.* The use of cocoa leaves is limited chiefly to the Andes regions of South America, from Colombia to Argentina. In regions where cocoaism exists, 25 to 30% of the total population are cocoa chewers. The number of coca chewers of South America may be estimated to be 8 millions. The amount of coca leaves annually consumed is, at least, 14,000 tons. The individual daily dose varies between 10 and 100 grams, with 30 grams as a mean value. The extraction of alkaloids during a period of coca chewing varies from 66% to 96%, with 86% as a mean value, in respect to the total amount of alkaloids contained in coca leaves. The total amount of alkaloids consumed varies from 80 to 400 mg., with 180 mg. as a mean value. There is no increase in tolerance to cocaine among the coca chewers. The differences between the amounts of alkaloids ingested by the addicts of short-standing and of long-standing use are very small. The daily dose

of coca leaves does not increase during the span of life of the addicts. The duration of the coca chewing habit is more responsible than the size of the daily dose as a cause of the chronic symptoms found among coca chewers.

Toxicity to rats of diallyl acetic acid and other compounds containing the allyl radical. ERNEST C. HAGAN (by invitation), GEOFFREY WOODARD (by invitation), and ARTHUR A. NELSON. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Rectal suppositories containing the bismuth salt of diallyl acetic acid have been held responsible for a number of deaths in children. Implications are that the acid is the responsible chemical. The oral LD50 of diallyl acetic acid (DAA) for rats is in the neighborhood of 630 mg/kg., diallyl phthalate 770, diallyl adipate 750, and allyl alcohol 60. Intravenously DAA has the same LD50 as by oral administration. Symptoms of poisoning by these compounds are depression, arching of the back, ruffled coat, and generalized weakness with anorexia. Deaths, particularly with DAA, are delayed, some occurring several weeks after acute dosage. At autopsy gross examination of the organs shows the livers and kidneys to be pale. In addition the livers present a nutmeg zppearance. Microscopically both organs show principally fatty degeneration which is generally more severe in the liver than the kidney. Data obtained from feeding DAA at 25, 50, and 100 ppm. for 6 months to Osborne Mendel rats demonstrate growth suppression in female rats at all levels, and in male rats at 50 and 100 ppm. Females show as much inhibition at 25 as at 50 ppm., while males at 25 ppm. show growth comparable to that of their control animals. The capacity for detoxification of pentobarbital and phenobarbital was studied in rats which had received 100 ppm. DAA for from 1–2 months. With each barbiturate hypnosis was more prolonged in treated animals than in their litter controls.

Correlation between toxicity by inhalation and physical properties of beryllium oxide. R. H. HALL, S. LASKIN, G. SPRAGUE and B. H. BROWN (introduced by H. C. Hodge). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* The toxicity of inhaled BeO appears to depend upon the physical properties of the material, which, in turn, are related to the method of preparation. The processes currently employed in the production of BeO involve calcining a beryllium salt at high temperatures. Dogs, guinea pigs, rabbits and rats have been exposed 6 hours daily for 12–15 days to an atmosphere containing approximately 85 mg/m³ of BeO aerosol. Even at this relatively high concentration, one grade of BeO appeared to be virtually nontoxic. This material was fired at 1350°C. and was composed of unit particles having an arithmetic mean diameter of about 0.7μ, with aggregates of low porosity (5.2%). A second grade of BeO gave definite evidence of toxicity. This material was fired at 1100°C. and comprised unit particles of about 0.5μ diameter, with aggregates of markedly greater porosity (35%). Two of 20 rats died during the exposure period, while 3 of 5 rats, held for observation following exposure, showed a marked increase in their leukocyte count due to an absolute increase of neutrophils and lymphocytes. One of 4 dogs showed a transient low blood oxygen tension following exposure, and the lungs of this animal which was killed terminally exhibited extensive lesions. Dogs and rats exposed to an aerosol of BeO produced by calcining $Be(NO_3)_2$ at 400°C. exhibited weight loss during exposure, 1 of the 2 dogs showed a significant reduction in blood oxygen tension, and 8 of 40 rats died. This material was made up of unit particles of about 0.4 microns mean diameter and aggregates of very high porosity (134%). The small sizes were confirmed by x-ray diffraction analysis which also revealed trace quantities of a foreign substance identified as silica. On heating of the material at 1000°C., grain growth occurred followed by the disappearance of the silica lines.

Effect of mercurial diuretics on the succinic dehydrogenase system of the kidney. C. A. HANDLEY. *Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Texas.* The succinic dehydrogenase activity of rat kidney cortex homogenate was inhibited 20–50% by the intravenous administration of non-lethal doses of mercurial diuretics. The dosage range used was 0.1 to 0.2 cc/kg. (4–8 mg/kg. of combined mercury), the range generally used for diuresis in laboratory animals. One hour after the injection, the kidneys, liver and heart were removed and 5% homogenates of each organ prepared for assay. In none of the experiments was the succinic dehydrogenase activity of heart or liver reduced below that of the controls. When the mercurial diuretic was added directly to Warburg flasks containing homogenates of kidney cortex, liver and heart from untreated animals, so that the concentration of mercury was 5×10^{-6} to 10^{-5} M, a comparable inhibition of succinic dehydrogenase activity occurred in all the homogenates. It is concluded that during mercurial diuresis, sufficient mercury is accumulated in the kidney cortex to partially inhibit the succinic dehydrogenase activity.

Amebacidal action of agents inhibitory to associated bacteria in vitro. EDER L. HANSEN (introduced by Hamilton H. Anderson). *Div. of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif. E. histolytica* has not yet been cultivated in the absence of bacteria. Agents that affect trophozoites may do so either directly or indirectly by acting on associated bacteria (Brackett & Bliz-

nick, 1947). It is now possible to detect indirect (bacteriostatic) action by modification of the conventional testing procedure, so that the intense reduction produced by associated bacteria is preserved. Such reducing conditions are necessary for survival and multiplication of trophozoites, since slight disturbance of bacterial growth can result in death of the ameba. Tubes containing dilutions of any agent found to be 'amebacidal' when tested by conventional procedure were sealed with melted petrolatum (Shaffer & Frye, 1948). Survival of trophozoites in sealed cultures, after 48 hours at 37°C., indicated that apparent 'amebacidal' action observed in cotton-stoppered tubes was due to inhibition of bacteria and not necessarily to direct action on the ameba. The method has been applied to Locke's egg slants and to the two liquid media of Hansen & Anderson (1948), using as bacterial associates organism *'t'* or a 2-membered flora. With the latter flora selective action of an inhibitory agent further complicated evaluation of amebacidal activity. Aureomycin HCl was 'apparently amebacidal' in liver-proteose-peptone medium at 1/500,000; however in sealed cultures, trophozoites survived in concentrations of 1/50,000. At higher concentrations organism *'t'* was so greatly inhibited that satisfactory reducing conditions were not established even in sealed cultures. Emetine HCl, which exhibited no bacteriostatic action, was amebacidal at the same concentrations in both cotton-stoppered and petrolatum-sealed cultures.

Comparison of effects of injection of pyrogenic solutions by intravenous and intramuscular routes. WILLIAM D. HARKNESS (by invitation) and BERT J. VOS. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Rabbits were injected either intravenously or intramuscularly with a pyrogenic solution prepared from Pseudomonas aeruginosa, and their rectal temperatures were observed at hourly intervals for 5 and 5 hours respectively. The rise in temperature following intramuscular injection was more gradual than that following intravenous injection and frequently had not reached a maximum even at 5 hours. Within certain limits a linear relationship existed between log dose of pyrogen and the maximum rise in temperature observed during the experimental period. However, at least 4 times as much pyrogen was needed by intramuscular as by intravenous administration to produce a given rise in temperature.

Pathologic changes in guinea pigs produced by diets deficient in the anti-stiffness factor. PAUL N. HARRIS and ROSALIND M. WULSEN (by invitation). *The Lilly Research Labys., Eli Lilly and Co., Indianapolis, Ind., and the Dept. of Zoology, Oregon State College, Corvallis, Ore.* It was found by Wulsen and Bahrs (Am. J. Physiol. 133: 500, 1941) that maintenance of guinea pigs upon pasteurized milk or skim milk diets resulted in stiffness of the wrists. Diets of skim milk powder dissolved in skim milk or water and supplemented with ferric chloride, copper sulfate and adequate amounts of the known vitamins have a similar effect, and were used in the studies herein reported. Hamsters kept upon such diets remained normal, but guinea pigs developed widespread anatomical lesions. These consisted in necrosis of skeletal muscle with a variable amount of macrophage infiltration and foreign body giant cell formation and calcification of necrotic fibers; focal necrosis and calcification of the myocardium; deposition of calcium salts in the smooth muscle of the gastrointestinal tract, and in the kidneys, liver, aorta and peripheral arteries; and development in the muscles and adjacent to bones and joints of abscesses that often became calcified.

Skin penetration of radioactive anticholinesterases—a direct quantitative method of study. E. ROSS HART, JOSEPH H. FLEISHER (by invitation), and AMEDEO S. MARRAZZI. *Toxicology Section, Medical Div., Army Chemical Center, Md.* Any study of the ability of a compound to penetrate the skin necessitates some means of determining its concentration in the blood. Chemical methods that are sufficiently specific and sensitive are often lacking. Moreover, they require repeated sampling thus disturbing the animal economy and providing only discontinuous determinations. Pharmacological methods are of limited usefulness because of their inherent lack of precision and their indirectness, latent periods, compensatory mechanisms etc., all introducing complications. Radioactive tracer methods offer the advantages of being highly sensitive and specific and, as utilized here, requiring no sampling, being essentially direct and continuous. Increased sensitivity and specificity can be attained by measuring the radioactivity of venous blood draining from the area of application thus avoiding dilution in the entire blood volume and minimizing the possibilities of chemical alteration between penetration and determination. The rabbit ear seems almost ideally suited to our purpose. By applying tagged compounds to the distal portions and placing a Geiger-Mueller tube over the proximal portions of the marginal vein, we have accomplished the conditions outlined above. Information concerning routes of penetration can be obtained from radioautographs of cross sections of the ear. The technique is described in detail and the results of its application to DFP are presented as an example.

Streptomycin content of brain and other tissues after parenteral injection. J. E. HAWKINS, JR., G. E. BOXER, and V. C. JELINEK (by invitation). *Merck Inst. for Therapeutic Research and The Research Labys. of Merck & Co., Rahway, N. J.*

The streptomycin content of blood, cerebrospinal fluid, brain, liver, lung and spleen was determined by the fluorometric method in 3 cats killed 24, 48 and 72 hours respectively after receiving a subcutaneous injection of streptomycin $CaCl_2$-complex equivalent to 400 mg. of streptomycin base/kg. of body weight. The blood concentration at 24 hours was too high for the tissue concentrations to be meaningful. At 48 and 72 hours, when the blood and cerebrospinal fluid concentration had fallen to 1–2 μg/ml., the viscera contained 9 to 38 μg/gm. (wet weight) but the brain only 2–4 μg/gm. This experiment shows that streptomycin tends to persist in the viscera when it has virtually disappeared from the body fluids, but has no special predilection for the brain. In a second experiment similar determinations were made in 4 cats which had received daily doses of streptomycin equivalent to 100 mg. of base/kg. for 7 to 28 days. All but one showed the characteristic vestibular disturbance. Blood and cerebrospinal fluid taken 24 hours after the last dose of streptomycin contained 1–3 μg/ml. and the brain only 1–2 μg/gm., while the concentration in lungs, liver, spleen and salivary glands ranged from 7 to 41 μg/gm., and in the kidneys from 119 to 234 μg/gm. The significance of these observations is discussed in relation to the therapeutic and neurotoxic actions of streptomycin. It is evident that the neurotoxic action does not depend upon a selective accumulation of the drug in the brain.

Pharmacology and toxicology of thiodipropionic acid and its dilauryl and distearyl esters. Lloyd W. Hazleton and Rebecca C. Hellerman (by invitation). *Hazleton Labys., Falls Church, Va.* Thiodipropionic acid ($S[CH_2 \cdot CH_2 \cdot COOH]_2$) and its dilauryl and distearyl esters have antioxidant properties of interest in the preservation of edible fats and oils. The acid is slightly soluble in water while the esters are insoluble over a wide range of temperatures. In rats, mice and guinea pigs the acute toxicity of these compounds is of a relatively low order of magnitude. Infused intravenously in anesthetized dogs the lethal dose of the acid is in excess of 1.0 gm/kg. The acid possesses no characteristic pharmacological properties and death in these animals is apparently due to hemodilution and acidosis. Two-year rat feeding experiments were essentially negative for the acid and distearyl ester in levels up to 3.0% of the diet. The 3.0% dilauryl level showed an increase in mortality toward the end of the period. In another series a mixture of the acid and dilauryl ester was heated in lard at 190°C. for 30 minutes. The diets as fed contained 10% lard plus 0.11% and 1.1% of the acid-ester mixture. At the lower level there was no significant effect on growth or mortality during the 2-year period. At the higher level there was significant mortality during the first year. The immediate cause of this increased mortality was respiratory infection. The possible role of the diet in this phenomenon was not established. Based on a 2-year expectancy, all the groups showed from 83% to 99% of the theoretically perfect score with the exception of the two groups discussed which showed 75% and 37% respectively.

A new adrenergic blocking agent. Francis G. Henderson (by invitation) and K. K. Chen. *The Lilly Research Labys., Eli Lilly and Co., Indianapolis, Ind.* A study was made with ethyl β-choloroethyl β-*o*-benzyl) phenoxyethylamine HCl. Its adrenergic blocking activity was found to be approximately 7.5 times that of dibenamine, but its toxicity greater than that of the latter, the LD_{50} in mice by intravenous injection being 33.81 ± 1.28 mg/kg. as against 88.45 ± 3.02 mg/kg. for dibenamine. A dose of 2 mg/kg. intravenously reversed the pressor response ordinarily produced by 25 μg. of epinephrine HCl in the dog under barbiturate anesthesia. When a dose of 5 mg/kg. was given intravenously the reversal action persisted for over 30 hours. A reversal of the pressor action of epinephrine also occurred 25 hours following the oral administration of this compound in the dose of 20 mg/kg. mixed in an acacia suspension. A dose of 5 mg/kg. intravenously prevented the rise in blood pressure commonly produced by intravenous injections of 1 mg. of the following aliphatic amines: 2-aminoheptane sulfate, 2-amino-4-methylhexane sulfate, and 2-methylamino-1-cyclopentylpropane HCl. No epinephrine reversal could be demonstrated on the cat's uterus. When administered to dogs by slow intravenous injection in doses ranging from 2 to 10 mg/kg., the compound per se caused no significant changes in blood pressure, respiration or electrocardiogram.

Effect of myanesin on extrapyramidal facilitatory and inhibitory systems. E. Henneman (by invitation), A. Kaplan (by invitation) and K. Unna. *Depts. of Psychiatry and Pharmacology, Univ. of Illinois College of Medicine, Chicago, Ill.* Hyperactivity of stretch reflexes due to overactive or unopposed extrapyramidal facilitation constitutes spasticity. Demonstration of the origins of separate spinal paths for facilitation and inhibition of myotatic reflexes in two distinct regions of the brain stem (Magoun) suggested investigation of the effects of myanesin on these systems. Bipolar stimulating electrodes carried in a stereotaxic apparatus were used to explore the bulbar reticular formation in lightly nembutalized cats. Regions yielding complete inhibition (medial, medullary sites) or satisfactory facilitation (lateral, pontine sites) of the knee jerk with low voltage were located. Myanesin was administered intravenously. Small doses reduced facilitation or inhibition. Larger doses abolished either effect, but left the knee jerk, as judged by amplitude, unaffected.

The two antagonistic systems were influenced equally so far as could be judged. The time course of drug action was determined by following the recovery of such systems. Inhibition and facilitation of the knee jerk resulting from cerebral cortical stimulation were abolished with much smaller doses, suggesting that longer or more complex circuits (such as are usually involved in spasticity) are more vulnerable to myanesin. Purely spinal facilitatory or inhibitory reflex arcs were also investigated by stimulating peripheral skin or muscle nerves in decapitate preparations. From this type of evidence and from electrical studies of segmental spinal reflexes it appears that myanesin relieves spasticity by reducing tonic extrapyramidal facilitation of stretch reflexes, whatever its source. Because facilitation dominates inhibition in spasticity it is presumably more affected by the drug.

Cardiovascular action of magnesium sulfate solutions. JAMES P. HENSEN (introduced by R. P. Ahlquist). *Dept. of Pharmacology, Univ. of Georgia School of Medicine, Augusta, Ga.* The cardiovascular actions of magnesium sulfate solutions, administered intravenously and intra-arterially, were investigated in the anesthetized dog. Arterial pressure was recorded by means of either an optical manometer or an ordinary mercury manometer while the femoral arterial or venous blood flow was recorded by a rotameter or 'Bubblemeter'. The results indicate that magnesium sulfate solutions lower the arterial pressure by two different mechanisms: *1*) peripheral vasodilation and *2*) myocardial depression. The peripheral dilation was found to be due to *1*) a direct action of the magnesium ion (an effect proportional to the dose administered) and *2*) some osmotic action (an effect proportional to the concentration of the injected solution). This osmotic effect was most pronounced with intra-arterial injections and could be duplicated by intra-arterial injects of hypertonic sodium chloride solutions. No evidence was obtained to indicate that the vasodilating action of magnesium sulfate was due to ganglionic blockade in a manner analogous to tetraethylammonium.

Pharmacologic properties of 2-[N-p'-tolyl-N-(m' - hydroxyphenyl) - aminomethyl] - imidazoline hydrochloride (C-7337), a sympatholytic. ELIZABETH A. HERROLD (by invitation), ANNE CAMERON (by invitation), ALFRED EARL (by invitation), FRANK ROTH (by invitation), JAMES SMITH (by invitation), NICOLINE SMITH (by invitation), ELIZABETH SORENSEN (by invitation), BRADFORD N. CRAVER. *Div. of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.* This drug was synthesized by Marxer and Miescher and reported by Meier and Yonkman (Federation Proc., this issue). Doses of 0.2 to 1.0 mg/kg. have produced 'epinephrine reversal' in dogs, cats, guinea pigs and rats. C-7337, in sufficient dosage, produced the hypotension characteristic of sympatholytics. Other augmentory actions of epinephrine negated by C-7337 in the same range of doses were: *1*) contraction of nictitating membrane and *2*) salivation in the cat. About twice the doses required for adrenolysis sufficed for sympatholysis. The spasmolytic effect of epinephrine upon canine Thiry-Vella loops and upon the feline uterus was unaltered by the drug. It produced complete relaxation of the nicitating membrane in respect to such drugs as Priscol and Privine whereas atropine produced only partial relaxation; this could be but partly explained by its very weak antihistaminic and anticholinergic activities. In Warburg experiments cardiac tissue from the cat was as sensitive to the inhibitory action of C-7337 on respiration as hepatic tissue from the cat or rat (100γ/ml.—minimal inhibitory concentration). The LD_{50} in rats is 55 mg/kg. intravenously. In the dog 125 mg/kg. orally produced depression, tachycardia, defecation, salivation, emesis and recovery; 6.0 mg/kg. intravenously produced the same symptoms and final recovery. Warren, Woodbury and Trapold (this issue Federation Proc.) will report an intensive study of the effects of its chronic administration to dogs.

A rapid method for the determination of inulin in blood and urine. AEME HIGASHI (by invitation) and LAWRENCE PETERS. *Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* Bacon and Bell (Biochem. J. 42: 397, 1948) used a modified Seliwanoff reaction, devised by S. W. Cole, to determine fructose in cadmium sulfate filtrates of blood. They mentioned its application to the determination of inulin. We failed to recover added inulin consistently from blood and urine by this method. However, when a trichloracetic acid (TCA) rather than a $CdSO_4$ filtrate was used, and temperature was controlled within 0.5 C, accurate recoveries resulted. To 2.0 ml. of plasma or urine filtrate (8.0% TCA), containing 7.5–30.0γ of inulin, is added 3.0 ml. of 0.15% resorcinol in alcohol (95%). With the tubes immersed in ice water, 3.0 ml. of conc. HCl, containing 7.5 mg. of $FeCl_3$/l., are added with shaking. The tubes are heated in a water bath for 20 minutes at 79.0-79.5 C. After rapid cooling, the orange-red color is read in a Klett colorimeter (54 filter). Standard solutions are analyzed with unknowns on all occasions. Plasma containing no inulin gives blank readings equivalent to 0.7–1.4 mg. %. Urine from subjects undergoing clearance studies is diluted beyond the point where blank values are obtained. Glucose produces additional color requiring corrective calculation only at high concentrations, such as those obtained during Tm determinations. Numerous recovery experiments have yielded a maximum error of 2.5%. Simultaneous inulin and creatinine clearance values for dogs agree well.

In one experiment the average I/C ratio for 9 consecutive clearance periods was 1.036 (range, 0.99–1.08; S.D. 0.032).

Lack of antagonism of methionine to barbiturates. J. EUGENE HILL (by invitation), P. L. EWING, G. A. EMERSON and MELVILLE SAHYUN. *Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas, and The Sahyun Laby., Santa Barbara, Calif.* The nutritional significance of methionine suggests a possible antagonism by it of agents detoxified in the liver. White mice treated with tolerated doses of 0.1–0.5 gm/kg. of DL-methionine intramuscularly or intraperitoneally were given repeated intraperitoneal doses of hexobarbital or thiopental at short intervals until approximately 50% of untreated controls died, or single intraperitoneal doses of the LD_{50} of pentobarbital or phenobarbital. In each case, a larger proportion of the methionine-treated mice died than of the controls. With pentobarbital, mice fasted 24 hours again showed no protective effect of methionine. Methionine either is present in excess even in fasted mice or else is not involved in the major degradation reactions of barbiturates in the liver.

A polarographic study of certain quinones. JAMES G. HILTON (introduced by C. L. GEMMILL). *Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville, Va.* The polarograph has been used as a qualitative and quantitative instrument in the study of certain quinones. The quinones examined were: hydroquinone; 2-methyl-hydroquinone; 2,3-dimethyl-hydroquinone; 2,3,-5-trimethyl-hydroquinone; 1,4-naphthoquinone; 2-methyl-1,4-naphthoquinone; 2-methyl-3-hydroxy-1,4-naph-thoquinone (Phthiocol); 3-hydroxy-1,4-naphthoquinone (Lawsone) and sodium 1,2-naphthoquinone-4-sulfonate. These quinones were studied both in bicarbonate and in glycolytic enzymatic mediums. All of the quinones showed definite half-wave potentials in the bicarbonate-carbon dioxide solution and gave diffusion currents which were in direct proportion to the concentrations. A detailed study was made of the half potentials of 2-methyl-3-hydroxy-1,4-naphthoquinone (Phthiocol) at various levels of oxidation of this compound. A close correlation was obtained between these potentials and the oxidation-reduction potentials previously described by Ball. (J. Biol. Chem. 106: 515, 1943). In the glycolytic enzymatic systems, the half potentials were shifted and the diffusion current heights were either reduced or totally missing. It is concluded from this work that the polarograph is an accurate instrument for determining the concentration of certain quinones of as low as 1×10^{-4} M concentration in a bicarbonate medium and is suitable also for determining oxidation-reduction potentials of these compounds in bicarbonate buffered solutions. From these results in the glycolytic enzymatic systems, it is concluded that the half potential shift was due to a change in the oxidative level of the quinone system and the diffusion current variations were due either to combination or to destruction of part or all of the quinone.

Acute toxicity of hydroquinone. HAROLD C. HODGE, ELLIOTT A. MAYNARD (by invitation) and JAMES H. STERNER (by invitation). *Div. of Pharmacology and Toxicology, Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, and Eastman Kodak Company, Rochester, N. Y.* The characteristics of acute hydroquinone poisoning have been observed. Dying animals did so promptly, those surviving 20 minutes almost invariably recovered completely. Convulsions were commonly observed; the heart frequently beat after respiration ceased. Estimations of the LD50 have been made for a number of species. Following intraperitoneal administration, mice, rats, guinea pigs and rabbits have similar LD50's. After oral administration, cats were most susceptible, rats and rabbits less so, and mice most resistant. Cats can, however, ingest approximately the lethal dose daily for nearly a month if the HQ is mixed in the diet. Fasting rats were twice as susceptible as fed rats to oral doses. The LD50 (orally to rats) of HQ in propylene glycol was considerably greater than in water solution. Rats were maintained for a period of one month on *a*) a common laboratory mixed diet and *b*) a commercial rat ration; the acute LD50's (oral administration) were identical regardless of previous diets. The acute toxicity of commercial photographic-grade hydroquinone was the same as that of a carefully purified sample when each was administered intraperitoneally.

Relationship of chemical structure to biliary concentration in experimental cholecystography. JAMES O. HOPPE and S. ARCHER (introduced by A. M. Lands). *Biology Div., Sterling-Winthrop Research Inst., Rensselaer, N. Y.* Replacement of one of the hydrogens from the methyl group of 4-amino-3,5-diiodotoluene by 2-substituted n-alkanoic, cycloalkyl-alkanoic or aryl-alkanoic acids yielded compounds which were excreted into the bile of cats following oral administration in sufficient amounts to make the gallbladder opaque to x-radiation. Optimum results were obtained with the n-alkanoic acid derivatives when the number of carbon atoms attached to the phenyl nucleus approached seven. The cyclo-alkyl-alkanoic acid derivatives were ineffective because of poor absorption. The most satisfactory compound was found among the aryl-alkanoic acid derivatives in the form of the sodium salt of β-(4-amino-3,5-diiodophenyl)-α-phenyl propionic acid (WIN 508–2). The density of the shadows produced by WIN

508–2 appeared to be approximately 1.5 times as great as those of iodoalphionic acid at comparable dose levels. Upon oral administration to both cats and dogs, WIN 508–2 appeared to be relatively non-irritating to the gastro-intestinal mucosa, readily evacuated from the gallbladder following a fatty meal and to produce a minimum of vomiting and diarrhea. The acute oral toxicity of the free acid in mice was less than that of iodoalphionic acid whereas that of the sodium salt, WIN 508–2, was slightly greater because of more rapid and efficient absorption.

Metabolic studies in beryllium patients. Joe W. Howland and Christine Waterhouse (introduced by H. C. Hodge). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* Systemic manifestations of weakness, easy fatiguability, anorexia, and weight loss have played an important role in the symptomatology of many patients with beryllium granulomatosis. Metabolic studies were carried out on 4 patients with beryllium poisoning in an attempt to determine whether or not these patients were able to utilize and store nitrogen on an adequate dietary intake. Three of the 4 patients studied were malnourished, had lost between 30 to 40 pounds in weight during their illnesses. Yet none was able to establish a significant positive nitrogen balance on diets adequate in calories and protein. Fecal nitrogen was high on all 3 patients. Under the anabolic stimulus of testosterone, one patient had a negligible nitrogen storage response; however, another stored moderate amounts of nitrogen under this regime. The implications of these findings particularly in reference to anoxia and to the systemic effects of beryllium poisoning are discussed.

Effect of quinidine in cyclopropane and cyclopropane-epinephrine induced arrhythmias. R. A. Huggins (by invitation), R. A. Morse (by invitation), D. W. Chapman (by invitation), C. A. Handley, and L. F. Schuhmacher (by invitation). *Depts. of Pharmacology, Medicine and Anesthesiology, Baylor Univ. College of Medicine, Houston, Texas.* The protective action of intravenous quinidine sulfate and lactate against the onset of epinephrine induced cardiac arrhythmias under cyclopropane anesthesia has been evaluated as to rapidity of onset, duration of action and minimum effective dose. The dosages used were 1, 5, 10, and 20 mg/kg. The intravenous administration of quinidine sulfate or lactate in all dosage levels tested markedly reduced the incidence of this type of cardiac arrhythmia. No difference was found between the two forms of quinidine. The onset of the protective action was rapid, less than 0.5 minute after the completion of the injection, and independent of the dosage. Injections of epinephrine up to 10 minutes after the administration of quinidine caused no arrhythmias, regardless of the size of the dose. After 10 minutes, the duration of the protecting properties of quinidine tends to vary directly with the size of the dose, lasting for an average of 24 minutes with 1 and 5 mg/kg., and 1 to 3 hours with 20 mg/kg.

Failure to demonstrate biological competition between hypnotic and non-hypnotic barbiturates. Walter W. Jetter, Robert P. Brittain and Mary K. Nuter (introduced by George L. Maison). *Dept. of Legal Medicine, Harvard Medical School, and The Massachusetts Dept. of Mental Health, Boston, Mass.* Sodium salts of non-hypnotic barbituric acid derivatives (BAD) as follows *1*) 5-monoethyl, B. A.; *2*) 5-monophenyl, B. A.; *3*) 5-mono sec. butyl, B. A.; *4*) 5,5-ethyl, nitropropyl, B. A.; *5*) 5,5-methyl (1 methyl butyl), B. A.; *6*) 5,5-methyl (1 methyl butyl) 4-imino, B. A. furnished in small quantities by Abbott Laboratories, North Chicago, were tested for their analeptic properties against depressant barbiturates in the following way. Twenty-seven rabbits were anesthetized by intravenous pentobarbital (40 or 50 mg/kg.) and 26 by phenobarbital (150 or 175 mg/kg.). Four rabbits died within less than one hour after receiving the depressant barbiturate alone. The surviving group of 49 rabbits contained 24 animals that received pentobarbital and 25 that received phenobarbital. Fifteen of these (9 pentobarbital and 6 phenobarbital) served as controls and received no treatment. Each of the remaining 34 animals then received intravenously one of the six BAD test compounds in dosage of 50, 100, or 150 mg/kg. Each compound was tested in at least 4 rabbits (BAD no. 5 was not tested against phenobarbital because of insufficient supply). It was clear that in no instance did the injection of the test BAD compound significantly decrease the depth or duration of the anesthesia caused by the previously injected depressant barbiturate. It was concluded that the BAD compounds tested were not successful biological competitors against pentobarbital or phenobarbital under the conditions of the experiment.

Effect of antimalarial drugs on anaerobic glycolysis (acid production) in tissue extracts. Ralph Jones, Jr. (introduced by Carl F. Schmidt). *Dept. of Research Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* A study was made of the effect of several antimalarial drugs on anaerobic production of acid from glucose by rat brain extract, in the system described by Utter, Wood and Reiner (J. Biol. Chem. 161 : 197, 1945). Pentaquine and Pamaquin produced significant inhibition of acid production when present in concentration of 5×10^{-6} M. Quinine, Quinacrine, Chloroquine and Paludrine produced no significant inhibition when present in concentration of 2×10^{-3} M. The system described by Utter *et al.* was modified in an attempt to localize the site of inhibition of acid

production. Inhibition of the same order of magnitude occurred when Hexose diphosphate was the substrate, when Fluoride is present in concentration of 0.02 M, and when arsenate is substituted for phosphate in the system. These results indicate that these compounds interfere with the conversion of Hexose diphosphate to Phosphoglyceric acid. Studies of the mechanism of this inhibition are in progress. The effect of twenty 8-aminoquinoline analogues of Pamaquine and Pentaquine on acid production in these systems was studied. Alteration of chemical structure modified the ability of these compounds to inhibit acid production, but there was little correlation between inhibition of acid production and antimalarial activity.

Effects of ions and enzyme inhibitors on breakdown of pentobarbital by rat liver slices. J. B. KAHN, JR. (introduced by J. M. Coon). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* Although it is well established that some barbiturates are detoxified by tissues *in vitro*, the nature of the process is not well understood. It was therefore decided to study the effects of various ions upon the action of rat liver slices on pentobarbital. The ions studied are known to be effective in activating or inhibiting enzymatic reactions of various types. Approximately 200 mg. of liver slices were placed in each of two 250-ml. Erlenmeyer flasks, *A* and *B*, each containing 90 ml. of Krebs-Ringer bicarbonate buffer with glucose. *A* was boiled for 5 minutes while *B* was maintained at 37°. Both were placed, in a water bath at 37°, and 10 ml. pentobarbital, 0.6 mg/ml. in Krebs-Ringer bicarbonate buffer added. Air was bubbled through for mixing and oxygenation. Ten-ml. aliquots were taken from each at 0, 30, 60, and 120 minutes, and analyzed for unchanged barbiturate by a modification of the method of Goldbaum (J. Pharmacol. 94: 68, 1948). Controls in normal Krebs-Ringer bicarbonate buffer with glucose showed 10% destruction of pentobarbital in 60 and 120 minutes in flask *B*, while flask *A* remained constant. Doubling the concentration of potassium and omitting calcium had no effect. The effects of high and low concentrations of Na, K, Ca, and Mg, and of heavy metals, iodoacetate, and cyanide are also reported.

Effect of acetate donors on creatinine levels in blood and urine in dogs. A. KANDEL (by invitation) and MAYNARD B. CHENOWETH. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* Five unanesthetized dogs were injected intramuscularly, and one intravenously, with monoacetin (glycerol monoacetate). Bladder urine was collected by catheter for a period of 10 hours. As a control, urine was collected over periods of the same length but without administration of monoacetin. The intramuscular injection of monoacetin resulted in a 25% increase in the total amount of creatinine excreted. An 80% increase was observed in the animal in which a 5% solution of monoacetin was given intravenously. Creatine was not excreted during control periods, but appeared in the urine only after monoacetin was administered. Both creatine and creatinine are excreted in greatest amounts during the last 2 hours of the collection period. Intramuscular injection of propylene glycol in one of the animals revealed no increase in urinary creatinine. A bilaterally nephrectomized dog was given 4 gm/kg. monoacetin intramuscularly in 1 gm/kg. doses every 2 hours. Blood collected at hourly intervals showed a steady increase in the creatinine level. A marked augmentation in creatinine accumulation was observed during the 9th and 10th hours. Further experiments are being conducted to establish the nature of the increase in production of creatine congeners by acetate donors.

Chronic effects of large doses of acetanilid in dogs and rats. ALEXANDER G. KARCZMAR (introduced by Theodore Koppanyi). *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* This is an extension of a study of acute toxicity of acetanilid (700 mg/kg.) in dogs (Federation Proc. 6: 341, 1947). Daily oral administration of 100 mg/kg. of acetanilid caused no withdrawal symptoms after 3 weeks (2 dogs). Administration of 150, 100, and 200 mg/kg. (2, 2, and 7 weeks; Payne, J. Pharmacol. Exper. Therap. 53: 401, 1935) was followed by daily methemoglobin cycles (duration: 8 hr.) with peaks of 25, 15 and 35% of total hemoglobin respectively. Discontinuation of acetanilid in 1 dog after 11 weeks caused no withdrawal symptoms. Increase of acetanilid in 3 other dogs to 400 (3 days) and 600 (4 days) mg/kg. gave a methemoglobin peak of 65%. These levels of acetanilid administration resulted ultimately in reversible hypochromic anemia. There was drowiness and analgesia, but no withdrawal symptoms. Tolerance did not develop since methemoglobin, lethal dose and the time of death corresponded to those of acute experiments (o.c.). Dogs killed during the chronic experiments showed earlier, bone marrow hyperplasia and reversible changes in the liver and, later, non-specific changes in the central nervous system, cortex and viceral organs (Hicks and Karczmar, to be published). Daily intraperitoneal administration to rats of $\frac{2}{3}$ of the lethal dose of acetanilid (up to 4 weeks) gave similar results with a methemoglobin peak at 35%. Withdrawal symptoms developed neither in dogs (contrary to Payne, o.c.) nor in rats independently of the administration schedule employed.

Duration of action of anticholinesterases in dogs. ALEXANDER G. KARCZMAR (by invitation) and THEODORE KOPPANYI. *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* Potentiation of pressor responses to

acetylcholine in atropinized dogs (pentobarbital sodium anesthesia) is used in this laboratory as a qualitative and quantitative assay of anticholinesterases (Science 106: 492, 1947). This assay lends itself to establishing the duration of action of antiesterases (maximally potentiating doses used throughout) since the animal survives under test conditions for any desired time. It was found by means of this method that physostigmine and neostigmine are short-acting antiesterases with a duration of potentiation up to 6 hours. A thiophosphate type of antiesterase, 0-0-diethyl p-nitrophenol thiophosphate, belongs here also with a 5-hour duration of action (Anat. Rec. 101: 89, 1948). A compound with an alleged specific action on pseudocholinesterase, dimethylcarbamate of (2-hydroxy-5 phenyl-benzyl) trimethylammonium bromide proved to be a short-acting (2 hours) true anticholinesterase. Organic phosphate esters are either long- or intermediate-acting. DFP belongs to the first category with complete extinction of potentiation of pressor effects after one week. Hexaethyl tetraphosphate (HETP) and tetraethyl pyrophosphate (TEPP, possibly active factor of HETP) cease to act after 12 hours. In all cases, antiesterase activity decreases rapidly at first and slowly after 50% of the effect disappears. Determination of antiesterase activity in terms of nicotinic effects of acetylcholine measures the biological reversibility of esterase inhibitors. It generally confirms the results obtained in vitro and seems free of errors due to dissociability of the esterase-antiesterase complex during *in vitro* procedures, (Goldstein. J. Gen. Physiol. 27: 529, 1944).

Relation between chemical constitution and estrogenic action in a group of azomethines. H. H. Keasling and F. W. Schueler (introduced by E. G. Gross). *College of Pharmacy and Dept. of Pharmacology, College of Medicine, State University of Iowa, Iowa City, Ia.* This work is based on the working hypothesis that estrogenic compounds should consist of a relatively inert molecular structure with two active hydrogen-bond forming groups located at an optimum distance of 14.5 Å units (measured on Fisher-Hirschfelder-Taylor Atomic Models). Since the 4,4′-dihydroxy derivatives of stilbene (—CH=CH—) and azobenzene (—N=N—) (Schueler, F. W., Science 103: 221 (1946) show an estrogenic response, a study of the intermediate type of linkage as represented by the azomethines (—CH=N—) was carried out. In order to investigate the importance of variations in hydrogen-bond forming power, substitutions were made in the 4,4′ positions of the basic structure, benzylidene aniline. All 16 possible combinations of the 4 substitution groups, hydrogen, hydroxyl, methyl, and methoxyl, were prepared and tested. The dihydroxy derivative best fulfills the requirements or the hypothesis; the methoxy derivatives represent blocking of the hydrogen-bond forming groups; and the methyl and hydrogen substitutions are groups of low or no hydrogen-bond forming power. The estrogenic tests were carried out using ovariectomized rats; 4,4′-Dihydroxy benzylidene aniline produced a full estrogenic response in 12.5 mgm. doses administered subcutaneously, and in 25γ doses intravaginally. None of the other derivatives produced an estrogenic response in 25-mg. doses given subcutaneously. An investigation of other aspects of the general pharmacology of azomethines as well as the substitution of other ring linkages for the azomethine linkage in 4,4′-dihydroxy-diphenyl derivatives (e.g., —CO—NH—; —CH_2—O—; —SO_2—NH; —CH_2—NH—; —S—S—; —CH(C_2H_5)—N(C_2H_5)—; —CO—CO—; —CH(OH)—CO—; and —CO—O—) is now in progress.

Liver as the major organ involved in the detoxication of thiopental by the dog. A. R. Kelly (by invitation) and F. E. Shideman. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* Previous work from this laboratory, using *in vivo* methods, has established the liver as the major organ involved in the detoxication of thiopental in 3 animal species (mouse, rat, and man). *In vitro* experiments have also demonstrated the ability of both rat liver slices and mince to degrade this thiobarbiturate. Evidence presented here implicates the liver as the major site of detoxication of thiopental in the dog. One approach employed was a comparison of rate of disappearance from plasma of thiopental in normal dogs and in animals with reduced portal blood flow (Eck fistula). In both groups, plasma levels declined at the same rate for the first 8 minutes following injection of 20 mg/kg. of the drug. At 2.2 mg. % the curve for the Eck fistula animals leveled off and fell very gradually until the righting reflex was regained at 6½ hours at a level of 1.29 mg. %. The normal curve dropped much more abruptly, the righting reflex returning 56 minutes after injection at approximately the same plasma level (1.2 mg. %) as in the Eck fistula group. The second experimental method consisted of a comparison of plasma decay curves in the heart-lung, heart-lung-liver, and heart-lung-kidney preparation. The heart-lung preparation (morphine-chloralose anesthesia) showed a very gradually falling plasma thiopental curve, so that, after 70 minutes, approximately 25% of the drug had disappeared; over the same period of time, in the heart-lung-liver preparations, 2½ times as much was removed. Preliminary experiments indicate that heart-lung-kidney preparations may degrade thiopental to some extent although not nearly as rapidly as heart-lung-liver preparations.

Effects of podophyllotoxin and picropodophyllin on intact intestine and salivary secretion of non-

anesthetized dogs. MARGARET G. KELLY (by invitation), ALDO P. TRUANT (by invitation) and PAUL K. SMITH. *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.* A comparative study has been made of the pharmacological effects of podophyllotoxin and picropodophyllin (isomeric compounds obtained from podophyllin) on the intact intestine of non-anesthetized dogs, with Thiry-Vella fistulae. At the same time salivary secretion was investigated. The activity of the freed loop of the intestine was recorded by the classic rubber balloon and tambour method. Both compounds were injected intravenously in the form of a fine suspension in saline. Fifteen to 30 minutes after injection of 0.5 and 0.75 mg/kg. of podophyllotoxin in dogs weighing 10 to 15 kg. an irregular decrease in amplitude of contractions occurred, with a slight decrease in tone which at times increased. The rhythmicity of contractions remained normal. Normal gut activity returned in 3 hours at the above dose levels. At a dose of 5 mg/kg. of picropodophyllin no effects were seen on the gut but salivation was increased. However, respiratory rate was intermittently interrupted by periods of dyspnea. When a dose of 7.5 mg/kg. was used a regular increase in the amplitude of contractions was demonstrated with an increase in tone. In certain experiments intermittent quiescent periods occurred. In all the experiments with picropodophyllin, at the latter dose level, perfuse salivation was seen which was blocked completely by 0.1 mg/kg. of atropine. A dose of 0.5 mg/kg. of atropine was not completely effective in blocking the gut effects of picropodophyllin. In a total of 20 experiments no catharsis was seen and no animals died.

Use of C^{14} in studies of metabolism of plant products. F. E. KELSEY. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* Some of the technical problems arising in a program of biosynthesis of radioactive drugs and their use in metabolic studies are discussed. A simple Geiger tube for the determination of the radioactivity of very weak samples is described. Briefly it consists of a bell-shaped tube, the open end of which is fitted with a sliding-tray arrangement for the insertion of the sample within the sensitive area of the counter. The counting gas, which flows continuously through the counter at atmospheric pressure, is helium saturated with ice-cold absolute alcohol. The available space on the sample holder is approximately 20 cm^2. The absence of a window and the position of the sample permits the counting of all betaparticles escaping from the sample. High sensitivity is essential for this work because of the dilution of the C^{14} in the process of the biological synthesis of the drugs by the plant and the further dilution by the C^{12} of the experimental animal used for the metabolic studies. Methods of handling the biological materials are given with illustrations from experiments on the metabolism of radioactive digitoxin.

Actions of calcium and tetraethylammonium ions on the rat diaphragm preparation. CHARLES J. KENSLER (introduced by McKeen Cattell). *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* The rat diaphragm preparation stimulated through its phrenic nerve at rates of 5 or 30 per minute failed rapidly when placed in a calcium-free Ringer's solution but not when in potassium-, magnesium- or phosphate-free Ringer. The threshold concentration of calcium required for minimal activity varied from 4.5 to 6.5×10^{-4} M while that for maximum activity varied from 7×10^{-4} M to 1.2×10^{-3} M. High concentrations (2.5×10^{-2} M) of calcium depress activity. Tetraethylammonium bromide (TEA) has been found to substitute for calcium on a molar equivalent basis in restoring maximum activity in calcium-deficient solutions providing the calcium concentration has been adjusted to that required for minimal activity. TEA did not substitute for calcium at subthreshold levels and did not appreciably lower the calcium threshold. The sensitivity of the diaphragm preparation to the curarizing agents D-tubocurarine chloride (DTC) and dihydrobetaerythroidine HCl (HBE) was greatly increased by lowering the calcium concentration. Neostigmine was not an effective antagonist of DTC at low calcium concentrations. TEA antagonized the action of DTC and HBE and to a lesser extent the failure produced by acetylcholine following treatment with DFP. Triethylphenylammonium chloride showed slight anticurare activity and calcium replacement value but triethylsulphonium iodide, triethylethanolammonium chloride, and dimethyldiethanolammonium chloride showed neither.

Reversible blocking action of DFP at the neuromuscular junction of the rat diaphragm preparation. CHARLES J. KENSLER and CHARLES BERRY (introduced by McKeen Cattell). *Depts. of Pharmacology and Anatomy, Cornell Univ. Medical College, New York City.* A study of the action of DFP (diisopropyl fluorophosphate) on the rat phrenic nerve-diaphragm preparation (Bulbring, Brit. J. Pharmacol & Chemotherapy 1: 38, 1946) has shown that in low concentrations (5×10^{-6} M) it increased the response of the rat diaphragm to nerve stimulation at rates of 5 and 30/min., whereas higher concentrations (5×10^{-3} M) prevented the response. The high concentration of DFP caused failure within 2 minutes but this was readily reversed as long as one hour later. The bath temperature was 38°C. Measurement of the phrenic nerve action potential and the response of the diaphragm to direct stimulation localized this blocking action of DFP at the neuromuscular junc-

tion. In the presence of low concentrations of DFP and after exposure to high concentrations, acetylcholine (1–2 micrograms/ml.) 'curarized' the preparation. The evidence obtained is inadequate to differentiate between the two most likely explanations for the reversibility of this block: *a*) that the neuromuscular block produced by DFP is due to acetylcholine accumulation and that at least a functionally adequate amount of cholinesterase recovers its activity or *b*) that DFP in high concentrations possesses an action affecting neuromuscular transmission other than cholinesterase inhibition.

Adrenolytic activity of a series of N-(9-fluorenyl)-beta-chlorethylamines. JAMES KERWIN (by invitation), GLENN E. ULLYOT (by invitation), EDWIN J. FELLOWS, EDWARD MACKO (by invitation). *Research Div. Smith, Kline and French Labys., Philadelphia, Penna., and Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Penna.* Substances with the following structure were compared with 'Dibenamine' for their effect after intravenous administration on the pressor action of epinephrine in cats anesthetized with pentobarbital sodium: $R_1—N(R)—CH_2—CH_2—Cl.HCl$. In all of the compounds R = the 9-fluorenyl radical. Activity greater than that of 'Dibenamine' was noted only in the case of the compound in which R_1 was ethyl. In those substances in which R_1 was methyl, propyl, isopropyl or secondary butyl, activity comparable with that of 'Dibenamine' was obtained. The derivatives in which R_1 was n-butyl, isobutyl, n-amyl, isoamyl, n-heptyl, benzyl or phenylisopropyl were less active than 'Dibenamine'.

Studies on radioactive digitoxin (*Digitalis lanata*). T. EUGENE KIMURA (by invitation) and E. M. K. GEILING. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* Radioactive digitoxin was biosynthesized by the incorporation of $C^{14}O_2$ into *D. lanata* by employing the method as reported by Geiling *et al.* (Science 108: 558, 1948) and by the concurrent use of a humidity-controller recently described (Science 108: 560, 1948), while extraction of the glycoside was conducted in a manner substantially similar to that described by the authors. Quantities of $C^{14}O_2$, varying from a total of 600–1440 uc (300–180 uc/plant) were introduced in divided doses at suitable intervals into batches of lanata grown for 4–6 weeks. Intensities of radioactivity from the crystallized glycosides in the order of 15,000 cpm/mg. and 25,000 cpm/mg. were registered from a 10-cm.^{2} surface, employing an internal Geiger counter (to be described elsewhere) when plants given 180 uc and 300 uc, respectively, were employed. Preliminary studies on the perfused hearts of rats and guinea pigs indicate presence of higher levels of radioactivity localized in the ventricles than in the atria, the rat heart showing a greater degree of difference than the guinea pig. Distribution studies have been carried out in the frog, guinea pig, and rat.

Further studies on the pharmacology of adrenolysis. THEODORE C. KING (introduced by Theodore Koppanyi). *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* The hemodynamic responses of nembutalized dogs to epinephrine, DL-nor-epinephrine, and DL-N-isopropylarterenol (isuprel) (5–10 gamma/kg.) were studied following administration of gradually increasing doses of yohimbine, benzylimidazoline (priscol), dibenamine, and ergotamine. Epinephrine effects are progressively decreased until complete abolition of the pressor response is obtained at the following dosage levels (mg/kg.): yohimbine—0.75, priscol—2.0, dibenamine—1.0. Reversal of epinephrine pressor effects does not occur until larger doses of these compounds are given (mg/kg.): yohimbine—2.0, priscol—10.0, dibenamine—10.0. Diminution but never complete inhibition or reversal of nor-epinephrine responses was observed with all doses cited. Fifty per cent diminution of response to nor-epinephrine was observed with yohimbine—0.2; priscol—0.4; dibenamine—3.0 (mg/kg.). Isuprel effects showed 50% inhibition only following yohimbine (0.5 mg/kg.) and priscol (1.0 mg/kg.). Isuprel effects were exaggerated by dibenamine. Ergotamine tartrate (0.05 mg/kg. or more) produced well over 100% potentiation of epinephrine and nor-epinephrine pressor effects. Similar doses produced complete reversal of isuprel effects with pressor responses over 50 mm. Hg. Epinephrine responses potentiated by ergotamine are reversed only by larger than the minimum effective doses of priscol or dibenamine.

Pharmacological actions of two aliphatic amines. THEODORE O. KING (introduced by Theodore Koppanyi). *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* Cocaine HCl (5–10 mg/kg.) administered intramuscularly completely abolishes or greatly inhibits pressor responses elicited by 2-methyl amino-6-hydroxy-6-methyl heptane (aranthol, Bilhuber-Knoll) (1.0–5.0 mg/kg.) and by 2-methyl amino heptane (oenethyl, Bilhuber-Knoll) (0.02–0.10 mg/kg.). In similar experiments in which dibenamine HCl (20 mg/kg.) was used, complete abolition or marked (65–95%) inhibition of the pressor responses to aranthol and oenethyl were observed. Cocaine was generally more effective than dibenamine in this respect so that when cocaine was used following dibenamine, further depression of the reduced responses were obtained. In experiments in which dibenamine was used following cocaine, small pressor responses were

obtained with aranthol after dibenamine administration, even in cases where there had been complete abolition of pressor responses by cocaine. The effectiveness of aranthol in antagonizing hypotension due to spinal anesthesia (procaine) was tested in laminectomized dogs. Aranthol HCl (5 mg/kg.) was administered after the mean arterial blood pressure fell to about 80 mm. Hg. The injection of this amine produced pressor effects up to 50 mm. Hg. The blood pressure then gradually fell to a level still 10 mm. above the hypotensive minimum and remained at this level for 1¾ hours. Aranthol was also effective in dilating the pilocarpine-constricted bronchial musculature. No tachyphylaxis was observed when aranthol HCl (2.5 mg/kg.) was injected intravenously at 5-minute intervals.

Heart sounds—electrostephone and recording machine (motion picture). HARRY KIRSCHBAUM (introduced by F. F. Yonkman). *Detroit, Mich.* This presentation deals with amplification and registration of cardiac sounds. It includes a description of the electrostephone and recording device. This device amplifies human fetal heart sounds and a permanent recording of the fetal and adult heart sounds can be made on wire, tape or film. By means of this instrument the response can be determined of both fetal and adult hearts to various pharmacologic agents. The machine has been used successfully in experimental research as well as in clinical surgery.

Cholinesterase as a cholinergic blocking agent. THEODORE KOPPANYI and ALEXANDER G. KARCZMAR (by invitation). *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* Among the cholinergic blocking agents, atropine-like and curare-like drugs owe their activity to specific effects on acetylcholine receptors. They appear to act independently of the acetylcholine-cholinesterase chain reaction; in fact, some show an antiesterase rather than an anti-acetylcholine effect. The employment of a new purified, injectable, true cholinesterase (Special Chemicals Division, Winthrop-Stearns, Inc.) added a third type of cholinergic blocking agent to pharmacological technique (Anat. Rec. 101: 36, 1948). This substance, upon intravenous injection, antagonizes both the muscarinic and nicotinic effects of injected acetylcholine in the intact mammal. The injection of cholinesterase into the turtle heart accelerates the rate and reverses other cholinergic effects obtained following the intracardiac injection of acetylcholine or of physostigmine. Cholinesterase injections, however, failed to prevent the cardiac effects of the faradic stimulation of the vagus. In cases where bradycardia produced by acetylcholine or physostigmine was abolished by cholinesterase, the vagus could not be paralyzed even by the intracardiac administration of massive doses of this enzyme. The effect of cholinesterase on injected acetylcholine, and the comparative inefficiency of the enzyme in relation to vagus stimulation were observed also in dogs and cats. Unless it can be demonstrated that the enzyme does not penetrate to the site of acetylcholine liberation in the neuro-muscular effector system, the above observation is of distinct theoretical significance.

Mechanism of action of anticholinesterase. THEODORE KOPPANYI. *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* Anticholinesterases potentiate the muscarinic and nicotinic effects of acetylcholine, the ganglionic effects of nicotine and the responses to cholinergic (including preganglionic) nerve stimulation. In larger doses they paralyze the sympathetic ganglia. This phenomenon is possibly independent of acetylcholine accumulation, because *a*) paralytic and antiesterase concentrations of individual drugs differ; *b*) nicotine pressor effects and pressor effects obtained on preganglionic stimulation are likewise abolished by paralytic doses of anticholinesterases; *c*) in some instances the paralytic effect of one antiesterase may be reversed by another, or even by the same anticholinesterase (Science 106: 492, 1947). This latter effect may be related to the reversal by anticholinesterases of nicotine paralysis of autonomic (not solely sympathetic) ganglia. Antiesterases, however, differ in several respects from each other. *a*) Those of the physostigmine or neostigmine type prevent the pharmacological effects of DFP (intact animal). Acetylcholine pressor effects in atropinized and physostigminized animals are not further increased following administration of various doses of DFP. On the other hand, DFP and other phosphate esters have no such inhibitory effects on physostigmine or neostigmine, or on each other. *b*) The antiesterases differ as to potency, ganglionic paralytic dose, duration of action, and as to shape of curves, expressing the relationship between their concentration and the magnitude of the pressor effect of acetylcholine in atropinized animals. *c*) Some antiesterases appear to inhibit pseudo-cholinesterase but not true esterase. The assay of anticholinesterases used in this laboratory did not confirm this specificity. Their action is better explained in terms of the concept of 'essential cholinesterase' (Milit. Res. Developm. Board 4: 271, 1948; Bull. Johns Hopkins Hosp. 83: 532, 1948).

Pharmacologic and toxicologic data concerning diethylaminoethyl ester of 1-phenyl-cyclopentane-1-carboxylic acid hydrochloride (Parpanit). CHARLES P. KRAATZ (by invitation), CHARLES M. GRUBER, JR., HUBERT L. SHIELDS (by invitation) and CHARLES M. GRUBER. *Dept. of Pharmacology,*

Jefferson Medical College, Philadelphia, Penna. The LD_{50} of parpanit has been determined as follows: rabbits intravenously, 24.5 mg/kg.; white mice intravenously, 45.1 mg/kg. white mice intraperitoneally, 222.3 mg/kg.; white rats intraperitoneally, 209 mg/kg. Toxic doses of the drug in these animals produce symptoms indicative of strong stimulation of the central nervous system, with death resulting ultimately from respiratory paralysis. In relatively non-toxic doses, parpanit has been found to have the following actions in experimental animals: a transitory vasodilatation independent of the central nervous system; a block of the terminal receptors of the parasympathetic-innervated structures rendering them insensitive both to nerve stimulation and applied acetylcholine; inhibition of activity of the intact intestine and depression of intestinal musculature; depression of the frog ventricle and excitation of the mammalian uterus. Comparative experiments have established that parpanit is similar qualitatively to trasentin which it resembles chemically, but is somewhat more active in inhibiting the intestine and in its parasympatholytic effect. Parpanit in the latter action is much less potent than atropine.

Effect of intravenous 'Tween' solutions in various animals and man. JOHN C. KRANTZ, JR. and JOSEPH G. BIRD. *Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore, Md.* Polyoxyethylene derivatives of sorbitan esterified with fatty acids such as oleic, lauric and stearic, are used as dispersing agents for fatty substances under the name of 'Tweens.' We have observed that these substances when injected intravenously into dogs (in dilute solution) and other species of the canine family, produce a prolonged fall in blood pressure, which presumably is due to the release of histamine or some histamine-like substance. In other species of common laboratory animals this condition does not obtain, nor has it occurred in a limited number of tests in man.

Uptake of bromsulphthalein by the liver of the rat. II. Studies with radioactive bromsulphthalein (BSP). JOHN KREBS (by invitation) and RALPH W. BRAUER. *Dept. of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans, La.* Bromsulphthalein containing S^{35} has been prepared by the reaction of phenoltetrabromphthalein with H_2SO_4 containing S^{35}. The material has been purified by chromatographic adsorption; one milligram of the end product contains 0.1 millicurie of S^{35}. The rate of exchange with inorganic sulfate of the $-S^{35}O_3$ attached to the dye in aqueous solution, in tissue slice experiments, and *in vivo* is unimportant quantiatively if the experiments are completed within 3 hours or less; the exchange rate under these various conditions being of the order of 5% per hour or less. Radioactive BSP has been used in a study of the release of BSP taken up by liver slices against very low concentrations of BSP in the circumambient fluid. Also the exchange of tagged BSP taken up by liver slices against various BSP concentrations in the medium has been studied. Results to-date indicate that the BSP taken up by liver slices falls into two fractions, one of which is released or exchanged at a rapid rate, while the second fraction, larger in amount, is released at a constant rate independent of the intracellular concentration. The dye was administered intravenously to a series of rats whose abdomen was prepared for quick laporatomy. Five minutes after the administration of the dye the livers were frozen by liquid air, transferred to a freezing microtome, and sections cut for radioautographic studies in a cryostat at −20°C. A stripping film technique was used for the preparation of the radioautographs. Results to-date confirm early conclusions that the uptake of BSP does not involve the Kupffer cells but proceeds directly from the plasma into the hepatic cells.

Quiescence preceding peristaltic constriction in the ileum. HUGO KRUEGER. *Dept. of Zoology, Oregon State College, Corvallis, Ore.* Subcutaneous injection of morphine and its derivatives in adequate doses usually initiates peristalsis in Thiry-Vella loops of the ileum in dogs. Activity of the lower ileum was recorded once weekly from a Brodie bellows connected to a tandem, double-chambered, water-filled balloon inserted into the lumen of the loop. The pressure used to distend the chambers of the balloon was 31 cm. of water. Drugs were administered after a 100′ control period. The advancing constriction of peristalsis, as described by Bayliss and Starling, is preceded by an advancing area of inhibition. In our experiments, the inhibition usually involved both a relaxation of the ileal muscle and a complete cessation of rhythmic contractions. Occasionally rhythmic contractions did not cease but were reduced in magnitude. The duration of the period of complete quiescence was 10 seconds or less in 731 out of 3057 measurements obtained from 2012 waves recorded during 536 experiments on 33 animals; 1453 waves had a period of complete quiescence between 10 and 30 seconds, 744 between 30 and 60 seconds and 129 were over one minute in duration. The longest period of quiescence measured was 290 seconds. Thus a quiescent period was questionable or negligible in 24% but was easily appreciable in 76% of the observations. The quiescence usually developed while the ileal muscle relaxed prior to the arrival of the advancing area of constriction but quiescence, prior to the arrival of the constricting ring, often occurred without relaxation of the ileal muscle, especially if the ileum was already nearly maximally relaxed.

***In vivo* action of morphine, urethane and pheno-**

barbital on the glycogen synthesis from glucose in the rat liver. ERNEST KUN and L. G. ABOOD (introduced by E. M. K. Geiling). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* Starved rats were injected with varying doses of morphine, urethane and phenobarbital one hour before glucose administration by stomach tube, and the liver glycogen and carbohydrate intermediates were measured 5 hours later. While normal starved rats synthetized an average of 36 mg. glycogen/gm. of liver in 5 hours after glucose administration, 100 mg/kg. morphine or 1 mg/kg. urethane completely inhibited glycogen deposition. Phenobarbital in 100 mg/kg. doses produced 58% inhibition. The degree of inhibition was a function of the dose. Morphine proved to be the most potent inhibitor, 10 mg/kg. causing 90% inhibition. Analyses for phosphorylated carbohydrate intermediates in drug-treated rat livers revealed a complete inhibition of esterification of inorganic P. Inorganic P was completely esterified in normal animals receiving glucose while none was esterified in the drug-treated ones. The quantitative distribution of the intermediates varied depending on the drug studied. A marked decrease in nucleoprotein phosphorus (0.1–0.2 mg/gm. compared with the normal of 1.5 mg/gm.) and phospholipid phosphorus (0.075–0.150 mg/gm. compared with normal values of 0.9–1.1 mg/gm.) was observed in the drug-treated animals.

Guanidine: the agent responsible for the convulsive response to intracisternally injected streptomycin. S. KUNA (by invitation), F. CUCHIE (by invitation) and H. MOLITOR. *Merck Inst. for Therapeutic Research, Rahway, N. J.* In an attempt to determine whether the convulsive response caused by streptomycin on intracisternal injection in rabbits was due to an intrinsic property of the streptomycin molecule, or to an impurity, the following investigation was undertaken. Testing individual components of this molecule indicated that guanidine was itself capable of causing this response. It was further demonstrated that streptomycin, streptidine or acetamidine caused like degrees of convulsive response when administered on the basis of their guanidine content. It was thus evident that the convulsive response produced by intracisternal administration is due to the guanidine content in the molecule. Impure streptomycin administered intracisternally on a unit basis caused more severe convulsions than the same amount of pure streptomycin similarly administered. Analyses of several lots of streptomycin of varying purity showed that on a unit basis, impure streptomycin contained a larger amount of guanidine than relatively pure streptomycin. When these lots were administered intracisternally in rabbits on the basis of their guanidine content, comparable convulsive responses were obtained. It is thus evident that the extra guanidine present as impurity or as degradation products in low potency streptomycin is responsible for the greater convulsive response observed following intracisternal injection of such material.

Storage of DDT in the fat of the rat. FRIEDA KUNZE (by invitation), A. A. NELSON, O. G. FITZHUGH and EDWIN P. LAUG. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Answers to the following questions were sought: *1)* Is there a level of DDT intake in the diet at which no storage in the fat occurs? *2)* What is the relationship between length of exposure to DDT and storage in the fat? *3)* How rapidly does DDT disappear from the fat after exposure is terminated? *4)* What is the relation between age and rate of DDT accumulation in fat? *5)* What is the lowest level of intake of DDT at which pathological changes may be seen in the liver? Answers to these questions are as follows: *1)* Accumulation of DDT in the fat occurs at every level of intake down to and including 1 ppm. *2)* With time, there is a progressive rise in storage, reaching a maximum at 23 weeks. This period of time necessary to reach maximum storage is independent of the level of intake within the range of 1–10 ppm. *3)* Fifty per cent of the DDT store still remains after one month on a DDT-free diet; 25% after 3 months. *4)* Comparison between groups of weanling and 4-month-old rats revealed identical rates of DDT accumulation, in spite of the fact that the consumption of DDT on a per kg. body weight basis is considerably greater in the younger animals. *5)* Evidence of liver injury has been noted in rats consuming diets containing 5 ppm. DDT for 4–6 months.

Beryllium posoning: II. One-year studies by intratracheal injection. C. W. LABELLE, P. MORROW, C. BOOTH, F. WESCOTT, G. MELVILLE (introduced by H. C. Hodge). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* A study has been made on a total of 325 rats and 18 rabbits exposed to beryllium, with suitable controls, plus smaller numbers of mice, guinea pigs, monkeys and dogs. Each of these animals received beryllium by intratracheal injection, usually as an insoluble compound, at levels ranging from 5 to 100 mg.Be/kg. Data are presented showing the resulting growth, mortality, hematology (10 variables), urine chemistry (15 variables), gross organ changes, organ weights (5 organs) and histological changes, and lung beryllium content over a period of one year after the injection. A chronic type of lesion developed in the lungs of most of the exposed animals which was consistent within a given species and for a given compound, although in no case identical with the human chronic lesion. During the same period, most of the commonly measured variables in the blood and serum remained normal.

The outstanding changes were increases in blood platelets and the absolute polymorphonuclear content of the circulating blood.

Bronchodilator action of butanol derivatives. A. M. LANDS, O. H. SIEGMUND (by invitation) and ESTELLE ANANENKO (by invitation). *Biology Div., Sterling-Winthrop Research Inst., Rensselaer, N. Y.* Bronchodilator action was determined in isolated perfused guinea pig lungs. The doses, in micrograms, required to abolish histamine constriction were: Butanefrine, 1-(3,4-dihydroxy-phenyl)-2-amino-1-butanol-120; isopropyl analog, 1 - (3,4 - dihydroxyphenyl) - 2 - isopropylamino -1-butanol HCl-5.0; cyclopentyl analog, 1-(3,4-dihydroxyphenyl) - 2 - cyclopentylamino - 1 - butanol HCl-1.0: epinephrine-5; Isuprel-1.5. Results obtained in guinea pigs with nebulized histamine are shown below:

Drug	Dose *mg/kg.*	Effect %
Butanefrine	0.5	78
Isopropyl analog	0.02	108
Cyclopentyl analog	0.05	115
Isuprel	0.01	118
Epinephrine	0.02	147

The cardiovascular action of these butanol derivatives is much weaker than that of Isuprel. The Isuprel ratios for action on heart rate were: Butanefrine—300, isopropyl analog—30, cyclopentyl analog—60. If the bronchodilator action is compared with the effect on the heart rate, the greatest therapeutic margin is obtained with the cyclopentyl derivative and with the isopropyl derivative > Butanefrine. The most effective bronchodilator derivatives are effective spasmolytic agents for the intestine and uterus. Acute toxicity was determined by intravenous injection into albino mice. The LD_{50} values in mg/kg. were: Butanefrine—117, isopropyl analog—57, cyclopentyl analog—84, epinephrine—2.7, Isuprel—77.

Alteration of the pH of the blood as a means of improving experimental cardiac anoxia. KURT LANGE, VICTOR TCHERTKOFF, FRANK GRAIG and DAVID WEINER (introduced by M. G. Mulinos). *Dept. of Medicine, New York Medical College, New York City.* The anoxic pattern found in E.K.G.'s of dogs and rabbits following exposure to lowered oxygen tension in the inspired air or to cold environment can be reversed by the administration of acids lowering the *pH*. Moreover a shift of the blood *pH* to the acid side prevents the appearance of the anoxic pattern of the E.K.G. of animals in an anoxia test. These effects are due to an increased oxygen dissociation with subsequent increase in oxygen available to the tissues. Normal animals do not show a change in their E.K.G. pattern with lowering of the *pH* of the blood within reasonable limits. They do show an anoxic pattern in the E.K.G. when their blood is shifted to the alkaline side. Calcium, phosphorous, sodium and potassium do not play a role in the reversal of the anoxic E.K.G. The *pH* shifts required to produce beneficial effects need only be slight and appear to be well within the range of safety.

Electroshock seizures in the hamster and their modification by antiepileptic drugs. EDWARD H. LANPHIER (introduced by Carl C. Pfeiffer). *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, Ill.* Application of electrical stimuli (60 cycle, 0.3 sec.) via electrodes placed in the buccal pouches of the hamster proved rapid and advantageous in producing convulsions. Individual mean thresholds ranged from 25.0 to 50.5 ma. with a mean of 36.5 ma. The average standard deviation in a series of daily determinations amounted to 6.3% of the average mean. The coefficient of correlation between weight and threshold proved to be +0.754, and useful predictions of threshold on the basis of weight could be made. Mortality in the hamster was 0.44% compared to 19% in the mouse. Clonic activity in maximal and sub-maximal seizures is much less prominent than in the mouse and rat, but modification of threshold and sizure pattern by antiepileptic drugs does not differ significantly. The hamster is thus a useful and convenient experimental animal for electroshock studies.

Effect of chemical configuration on the edema-producing potency (EPP) of acids, aldehydes, ketones and alcohols. P. S. LARSON, J. K. FINNEGAN and H. B. HAAG. *Dept. of Pharmacology, Medical College of Virginia, Richmond, Va.* The method used for measuring edema production is a modification of one that we have previously described (J. Pharmacol. 89: 115, 1947). Morphinized male albino rabbits are tied in a supine position. A pocket is made of the right eye upper lid and kept filled with a solution of the irritant for 3 minutes. One hour later the rabbit is killed, the upper palpebral conjunctiva of each eye excised, weighed, dried and reweighed. The ratio of moisture to dry weight for each membrane is calculated and the difference between the ratios represents the degree of edema production. For each irritant studied, the molar concentration needed to produce a 2.5 unit increase in moisture to dry weight is determined. With monobasic organic acids, excepting formic, EPP increases with increasing molecular weight. Corresponding dibasic acids may have a lower EPP while unsaturation may increase EPP. HCl has no greater EPP than the more weakly ionized monobasic organic acids. With saturated aldehydes, excepting formaldehyde, increasing molecular weight results in increasing EPP. Unsaturation may markedly increase the EPP but this effect decreases with increasing molecular weight. Insofar as solubility permitted testing, it

appears that with ketones the EPP increases with increasing molecular weight. Among primary alcohols, EPP increases markedly with increasing molecular weight, decreases with unsaturation, and appears to be unaffected by isomerism. In passing from primary to secondary to tertiary alcohols, the EPP progressively declines.

Species variation in the protective action of anesthesia against the acutely lethal effect of nicotine. P. S. LARSON, J. K. FINNEGAN, J. CONWAY BIBB (by invitation) and H. B. HAAG. *Dept. of Pharmacology, Medical College of Virginia, Richmond, Va.* The protective action of anesthetics against death from acute intravenous administration of nicotine was first noted by Franke and Thomas (J. Pharmacol. 48: 199, 1933), who found that the smallest dose that was generally fatal for etherized dogs was 5 mg/kg. as compared to 3 mg/kg. for unanesthetized dogs. Subsequently Gold and Brown (J. Pharmacol. 54: 463, 1935) found that after barbital slightly more nicotine is required to cause death in cats than in the normal animal, but that the increment is less than twofold. In the present report, we have extended these observations to include other species. Male albino rabbits, rats and mice were used. Two intravenous LD_{50} determinations for nicotine were made on each species; one on unanesthetized and one on anesthetized animals. At least 3 groups of from 6 to 25 animals were used in each determination. The rabbits were anesthetized with sodium amytal (45 mg/kg., intravenously); the rats and mice with dial (60 and 100 mg/kg. respectively, intraperitoneally). The results are summarized in the accompanying table. In rabbits, anesthesia raised the LD_{50} value for nicotine about 60%, which is in keeping with the slight increase reported for dogs and cats. However, in rats and mice, anesthesia elevated the LD_{50} values 10- and 13-fold respectively. Thus a marked species variation exists in the protective action of anesthesia on the acutely lethal effect of nicotine.

Species	$LD_{50} \pm$ S. E. for nicotine (mg/kg.)	
	Unanesthetized	Anesthetized
Rabbit	5.9 ±0.6	9.4 ±0.2
Rat	2.8 ±0.3	27.5 ±1.7
Mouse	0.55 ±0.01	7.1 ±0.7

Cutaneous administration of adrenergic blocking agents by ion transfer. JULES H. LAST, ARTHUR A. RODRIQUEZ (by invitation), and ISADORE PITESKY (by invitation). *Depts. of Pharmacology and Physical Medicine, Univ. of Illinois College of Medicine, Chicago, Ill.* The local adrenergic-blocking effects of N,N-dibenzyl-β-chloroethylamine (Dibenamine) and N-ethyl-N-(1-naphthylmethyl)-β-bromoethylamine hydrobromide (SY28) have been studied in rabbits. The drugs were applied to rectangular areas (1.25 x 12.5 cm.) of the skin of the abdomen and repelled through the skin by a current density of 0.33 milliamps/cm.^{2} applied for 5 minutes. SY-28 was applied in total doses of 0.02–0.4 mg., whereas Dibenamine was used in doses of 0.4–1.6 mg. so as to produce equivalent blocking. The intensity of vasoconstriction following the intracutaneous administration of epinephrine (1:1000–1:40,000) was used as the indicator mechanism to reveal adrenergic blocking activity. The penetration of both SY-28 and Dibenamine was expedited by ion transfer and resulted in adrenergic blocking action at significantly lower dosage levels. SY-28 was found to be 10 to 20 times as potent as Dibenamine under the conditions of these experiments. Evidence of the epinephrine-reversal phenomenon has been noted in the rabbit skin following the local or intravenous administration of both SY-28 (0.1–4.0 mg/kg.) and Dibenamine (4–32 mg/kg.). These observations reveal the presence of vasodilator fibers in the skin of the rabbit.

Chronic toxicity of phenylmercuric acetate and mercuric acetate in the rat. EDWIN P. LAUG, O. G. FITZHUGH, FRIEDA KUNZE (by invitation), and A. A. NELSON. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* At the end of a 2-year feeding experiment, in which phenylmercuric acetate and mercuric acetate were fed to rats at levels of 0.1 ppm. to 160 ppm. mercury in the diet, the following observations were made: *1*) Phenylmercuric acetate is considerably more toxic than mercuric acetate on the basis of mortality and growth effect. Thus reduction in growth was noted on 10 ppm. phenylmercuric acetate, but only at 160 ppm. mercuric acetate. *2*) Diets containing phenylmercuric acetate in the range of 0.5 to 10 ppm. mercury, support approximately 20 times the storage of mercury in the kidney than comparable levels of mercuric acetate. *3*) Phenylmercuric acetate produced gross effects on the kidneys, such as pitting and hypertrophy. These effects were evident at dietary levels as low as 2.5 ppm. *4*) Injury to the kidney tubules, more pronounced in the females than the males, could be demonstrated at a level of 0.5 ppm. for phenylmercuric acetate. This was $\frac{1}{20}$ of the level of mercury intake at which comparable damage could be demonstrated with mercuric acetate. *5*) Urinary excretion studies show that on diets containing phenylmercuric acetate the excretion of mercury is 10 to 20 times greater than that on diets containing mercuric acetate. Conversely, the fecal excretion with mercuric acetate is 3 times as great as with phenylmercuric acetate. These facts are taken to indicate that the increased toxicity of phenylmercuric acetate arises from its superior absorption into the organism.

A practical classification system for drugs. C. D. LEAKE. *Pharmacology Laby., Univ. of Texas Medical Branch, Galveston, Texas.* Aristotle emphasized the importance of classifying diverse concepts as a necessary prerequisite to their systematic consideration. Classification is accomplished logically by abstraction and analogy. Neither of these has been encouraged in the properly skeptical and critical development of modern experimental biology and medicine. With the tremendous accumulation of diverse factual concepts in such a science as pharmacology, a working classification is becoming increasingly necessary if the science is to be successfully taught or further explored. A classification of drugs on a chemical basis is not at present practical because the relationship between chemical constitution and biological activity is not yet satisfactorily developed, and does not broadly relate to the primary purposes of drugs. Such a classification of drugs as might be based on localization of biological activity, as proposed by H. H. Meyer, and later by Lamson, is also impractical because it is overly complicated, repetitive, and confusing in clinical practice. Indeed, the most satisfactory practical drug classification is one based on their chief use as a result of direct cumulative clinical experience. Such a classification is pedagogically helpful as has been well shown wherever it has been tried. It affords a systematic arrangement which aids the clinician and the researcher who may be trying to develop new drugs of clinical value or to improve on those already in use. Such a classification would conveniently follow clinical concepts in *1*) drugs used mostly for diagnosis of disease, such as halogenated phenolphthaleins for liver function tests; *2*) those directed most commonly to the prevention of disease, as vitamins, antiseptics, insecticides and rodenticides; *3*) those employed primarily for the cure of disease ('chemotherapy'), as quinine in malaria and penicillin in syphilis, and *4*) those drugs ordinarily found helpful in the alleviation of the symptoms of disease. For further convenience the latter may be subdivided into groups on the basis of the chief systems of the body involved with respect usually to stimulation or depression. Within these general groups further classification may advantageously be made on the basis of the mechanism of the biological action of the drugs concerned.

A warm wire analgesimeter. R. E. LEE (by invitation), H. L. WILLIAMS (by invitation), and CARL C. PFEIFFER. *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, Ill.* A simple apparatus to produce graded painful stimuli has been constructed. It consists of a stimulating point made of a 7 cm. length of 26-gauge nichrome wire, bent in half to form a sharp-angled tip, connected in series to a dry cell and a potentiometer of 2 ohms resistance and a D.C. ammeter graduated in 0.02 amperes reading up to 1.5 amperes. The temperature of the tip of the wire at ammeter readings of 0.4 to 1 has been calibrated with a thermocouple. Increases of 0.05 amperes result in an increase in temperature of 3 to 4°C. A temperature of 50°C. constitutes a painful stimulus for most individuals. By use of the stimulator on the forehead it has been possible to determine the rise in pain thresholds in human subjects treated with analgesic drugs. The instrument has also been used successfully to determine the degree of analgesia produced by morphine sulfate (5 mg/kg.) in mice. More quantitative results are obtained by this device than by the usual tail-pinch method. The device is also of teaching value to demonstrate delayed pain and to test for the duration of action of local anesthetics.

Pharmacology of thiomerin. ROBERT A. LEHMAN and E. E. KING (by invitation). *Dept. of Therapeutics, New York University College of Medicine, New York City.* Thiomerin (di-sodium salt of N-[gamma-carboxymethylmercaptomercuri, beta-methoxy] propylcamphoramic acid) is a mercurial diuretic obtained by replacement of the theophylline in mercuzanthin (Mercurophylline, *U.S.P.* XIII) by sodium mercapto-acetate to give a compound of the $-CH_2-Hg-S-$ type rather than $-CH_2-Hg-N<$. It was previously shown that in thiomerin (MT6) the acute cardiac toxicity characteristic of the conventional theophylline-bearing diuretics has been virtually eliminated at least with respect to the anesthetized cat. (Proc. Soc. Exper. Biol. & Med. 64: 428, 1947). The present report concerns other pharmacologic properties of thiomerin as contrasted with presently used drugs. Intravenous lethal doses in the mouse have been estimated for thiomerin, mercuzanthin, mercuhydrin and salyrgan-theophylline and were found to be of the same order of magnitude when observed over a 4-day period. However, within the first half hour after injection, thiomerin is approximately $\frac{1}{10}$ as toxic as the other 3 drugs. Similar ratios were obtained using the Langendorff isolated cat heart. Absorption was estimated by analysis of injected muscle in rabbits and the absorption curve for thiomerin appears to resemble closely the curves previously reported for mercuzanthin and salyrgantheophylline. (J. Pharmacol. & Exper. Therap. 62: 26, 1938). The rate and completeness of excretion of mercury after administration of the diuretics was studied in cardiac patients. No striking differences were found. Storage of mercury in the kidney and liver after chronic administration in experimental animals has also been investigated. Data will be included on the fate of the sulfhydryl group as well as that of the mercury and a mechanism will be suggested to explain the pharmacologic activity of a mercurial diuretic in which

dissociation has been suppressed by mercaptide formation.

Relative merits of commonly used sulfonamide drugs as components of mixtures. DAVID LEHR. *Dept. of Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.* At a daily dosage of less than 4 gm., even the 'worst offender' sulfathiazole did not usually produce sensitization (D. Lehr, Brit. Med. J. 2: 543, 1948). It follows that the renal protection of sulfonamide mixtures could be enhanced without the danger of increasing the incidence of allergic reactions, by using additional components in proportionately smaller amounts. It can be assumed as established that, at present, sulfadiazine and sulfamerazine qualify for first and second place, respectively, as mixture components. Sulfathiazole, sulfapyrazine, sulfamethazine and sulfacetimide deserve attention as possible 3d or 4th members. Sulfathiazole was eliminated from the U.S.P. because of its high clinical toxicity. However, mixtures containing partial amounts of this drug demonstrated a low overall toxicity. Sulfapyrazine, most insoluble of all compounds, induces frequent renal and allergic reactions and therefore offers no advantages over sulfathiazole. Sulfamethazine possesses adequate solubility and low toxicity but is subject to substantial conjugation and plasma binding which diminish its therapeutic efficiency. Sulfacetimide retains the high solubility of sulfanilamide and shares with the parent compound the improbability of renal concrement formation and the ready diffusibility into all tissues. It shows low figures for conjugation, plasma binding and sensitization. Recommended primarily against ocular and urinary infections, this drug demonstrates satisfactory bacteriostatic activity against a variety of human pathogens and gives good blood levels in man. In experimental animals, sulfacetimide proved about 5 times less toxic than sulfadiazine. The mixture sulfadiazine-sulfamerazine-sulfacetimide (equal amounts) revealed low toxicity in animals as well as excellent bacteriostatic activity in vitro and in experimental infections of mice. After oral administration, blood and spinal fluid levels were similar to those from equal doses of sulfadiazine or sulfamerazine. Preliminary clinical trials including several cases of pneumonia and two cases of penicillin resistant septicemia gave highly satisfactory results.

Sodium phthalyl sulfacetimide: A new gastrointestinal antiseptic. DAVID LEHR. *Dept. of Pharmacology, The New York Medical College, Flower and Fifth Avenue Hospitals, New York City.* Sodium phthalyl sulfacetimide (di-sodium N^1 acetyl N^4 phthalyl sulfanilamide di-hydrate) possesses good bacteriostatic activity against coliform organisms *in vitro* and in man. In addition it displays satisfactory activity against many bacterial pathogens of the intestinal tract, especially the Shigella group (H. Seneca and E. Henderson, personal communication). The compound is readily water soluble and stable in the alkaline range of the body. Spontaneous hydrolysis at the N^4 group remains negligible down to a *pH* of 6.0. A 20% aqueous solution of the drug has a *pH* of 7.30. After oral administration in rabbits, sodium phthalylsulfacetimide was present in aqueous solutions of 1500 to 3000 mg. % strength in the small and large intestines, whereas the equimolar amount of sodium sulfadiazine, as representative of absorbable sulfonamides, was dissolved in the enteric contents only to the extent of 70 to 400 mg. %. Yet absorption of sodium phthalylsulfacetimide into the blood stream was minimal although it diffused readily into the deeper strata of the intestinal wall and produced at this site concentrations more than twice as high as the equimolar dose of sodium sulfadiazine. From parenteral routes of administration in rabbits and rats sodium phthalylsulfacetimide readily entered the blood stream and was rapidly eliminated by the kidneys, causing high urinary concentrations. The intraperitoneal toxicity was surprisingly low (approximate LD_{50} for albino rats being 10 gm/kg. body weight). In man a single oral dose of 10 gm. sodium phthalylsulfacetimide did not produce any demonstrable blood levels and only about 1% of the dose appeared in the urine within 24 hours. Therapeutic trials in acute gastrointestinal infections seemed encouraging and deserve further exploration.

Tolerance in rats to new synthetic analgesic drugs. JOHN R. LEWIS (introduced by A. M. Lands). *Biology Div., Sterling-Winthrop Research Inst., Rensselaer, N. Y.* The development of tolerance to the analgesic action of morphine and several compounds chemically related to Demerol or methadone was followed in rats. The compounds investigated and respective doses used were morphine sulfate (10 mg/kg.); *l*-methadone hydrochloride (2 mg/kg.); *l*-isomethadone hydrochloride (3 mg/kg.); ketobemidone or 1-methyl-4-(3-hydroxy-phenyl)-4-piperidyl ethyl ketone hydrochloride (3 mg/kg.) and a sulfone derivative, *l*-ethyl 1,1-diphenyl-3-dimethylaminobutyl sulfone hydrochloride (3 mg/kg.). Data from a group of 20 rats were obtained for each compound. These rats were given daily subcutaneous injections for 6 weeks and their analgesic reactions and body weights determined. Tolerance developed most rapidly to the sulfone derivative and least to *l*-isomethadone. The development of tolerance to all the other compounds was quite similar and at the end of 6 weeks of daily administration the analgesic response was found to be only about 40% of the initial value. As tolerance developed there was a decrease in duration of action which was probably related to the decrease in maximum effect. There

was a general change in the character of the side-effects from depression to excitation following drug injection during the development of tolerance. After withdrawal, the only symptom observed was hyperexcitability which reached its maximum in about 48 hours. Recovery from the tolerance was least with ketobemidone and the sulfone derivative. With the other compounds, responses of about 90% of normal were obtained at 3 weeks after withdrawal. The daily administration of these compounds caused a decreased rate of growth which was probably due to a decrease in food consumption. Morphine caused the greatest and ketobemidone the least retardation of growth.

Screening test for radiopaque drugs. A. LIGHT (by invitation), P. B. DEWS (by invitation), J. TORNABEN (by invitation), E. H. LANG (by invitation), STATA NORTON (by invitation), and EDWIN J. DE BEER. *The Wellcome Research Labys., Tuckahoe, N. Y.* The methods currently used for the screening of compounds as possible cholecystographic agents usually require comparatively elaborate experiments on such animals as rabbits or dogs. Consequently, only limited numbers of animals may be studied. A convenient test employing the albino mouse has been developed. This test permits the use of large numbers of animals and the technique is simple because the mouse gall-bladder is not embedded in the liver and may be removed easily. Furthermore, only small amounts of drug are required. In performing the test, groups of mice are dosed orally with the drug being studied and, after a suitable time interval, are anestherized with Evipal and killed. The gall-bladders are removed, pooled and dissolved in alkali, and the iodine is determined chemically. Dosage-response curves with standard drugs were obtained and the test has been employed for routine assays. The method has been adapted to other problems such as the screening of iodinated compounds for possible pyelographic properties.

A method for rapid graphic solution of time-percentage effect curves. JOHN T. LITCHFIELD, JR. *Chemotherapy Div., Stamford Research Labys., American Cyanamid Company, Stamford, Conn.* A method for rapid graphic solution of time-per cent effect curves has been developed. The method is derived from the provisional graphic solution described by C. I. Bliss (Annals Applied Biol. 24: 815, 1937). In his method, times are converted to logarithms and cumulative percentage effects to probits. These data are plotted, a straight line fitted by eye, and the parameters and errors of the line obtained by means of formulae given. Although this method of solution is relatively easy in the case of a complete curve (all individuals reacting), it becomes much more difficult when the curve is incomplete due to failure of some individuals to react. In the new method, which uses the data in original form, and thus avoids the log-probit transformation, the data are plotted on logarithmic-probability paper; a straight line is fitted and the parameters are obtained from this line. Confidence limits of the parameters for 19/20 probability are obtained without computation by means of nomographs. When the curve is complete, only one of the nomographs is needed but when the curve is truncated, two additional nomographs are used. A complete solution may be made in any case in less than 5 minutes, without the use of a slide rule or calculating machine. The results are equivalent in all respects to those obtained by the more complex basic method. The method and nomographs are presented with several examples of their use.

Comparative toxicities of five substituted glycerol ethers. PRUDENCE C. LOEB (introduced by Charles H. Hine). *Div. of Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco, Calif.* Systemic toxicity after intragastric, respiratory and percutaneous absorption and topical effects from application to the eye and skin, and sensitization of skin were studied. The acute, intragastric LD_{50}'s (ml. or gm./kg.) in inbred white mice were: α-Ethyl (I) 9.35 $\pm$ 0.134; α-Isopropyl (II) 8.2 $\pm$ 0.135; α-Allyl (III) 4.2 $\pm$ 0.084; α-Phenyl (IV) 2.65 $\pm$ 0.025; α,γ-Diisopropyl (V) 1.86 $\pm$ 0.058. Loss of righting reflex followed by reversible muscular paralysis was noted with all the compounds. Non-paralyzing doses produced no sensory depression. Death followed depression of respiration, probably of central origin. The vapor pressures are extremely low, and exposure of mice to vapor-saturated air at room temperature 8 hours daily for 10 days produced neither irritation nor significant mortality. Mist exposure for one hour produced slight temporary respiratory irritation in hamsters. Only V produced systemic toxicity after cutaneous application. II produced mild primary irritation of intact shaved rabbit skin and moderately severe irritation of abraded skin; III and V caused mild primary irritation of abraded skin. Both alcohol and glycerine produced greater irritation. V caused mild erythema of rabbit skin after seven daily applications of 2 ml./kg. Twenty such applications of I, II, and III were not irritating. Similar applications of 10 ml./kg. (5% aqueous solution) of IV discolored the skin of rats, but did not cause irritation. II and V each caused mild transient irritation when instilled into the rabbit's eye; III caused moderate irritation. Guinea pigs were not sensitized by any compounds. The acute toxicity of the glycerol ethers is greater than that of the glycols.

Comparison between individual sensitivities to histamine and to diphenhydramine. S. LOEWE, L. A. WOODBURY (by invitation) and SUSANNE LOEWE PUTTUCK (by invitation). *Depts. of Phar-*

macology and Physiology, Univ. of Utah School of Medicine, Salt Lake City, Utah. When male guinea pigs inhaled nebulized histamine under uniform conditions, individuals differed little in 'histamine time' (T_H = inhalation time to a set endpoint). In 3 populations totaling 228 guinea pigs, mean T_Hs were 79, 80 and 83 seconds, with coefficients of variation (C.V.) of 12, 23 and 17%. T_H did not vary consistently with body weight. When, however, the I.P.D.$_{50}$ of diphenhydramine [median individual preventive (intraperitoneal) dose prolonging T_H to 390 seconds in 50% of experiments] was determined, individuals varied greatly (mean I.P.D.$_{50}$ of 75 animals: 6.6 mg/kg.; C.V. 68%). Variation with body weight was also great; with body weights increasing from 230 to 550 gm., mean I.P.D.'s rose from 2.6 to 8.9 mg/kg. In contrast, each individual's dose-response curve approximated an all-or-none curve. *1)* Sensitivities to histamine and to the antihistaminic are incongruous; this challenges the assumption of the same receptor substance for agonist and antagonist. *2)* Each individual is constant, but different individuals vary greatly in diphenhydramine response. This strongly advocates the use of intraindividual methods (Science 106: 89, 1947) of antihistaminic potency evaluation, to avoid the large standard errors of the group P.D.$_{50}$ assays. For example, in intraindividual assays, C.V.'s of the potencies (diphenhydramine = 1.0) of tripelennamine (5.18), C-5581 H (1.75) and Antistin (0.48) were 2.7, 5.4 and 4.8%, respectively.

Circulatory and other actions of sparteine in mammals. Go Lu (introduced by P. J. Hanzlik). *Dept. of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco, Calif.* The predominant effects of sparteine sulfate (5-20 mg/kg.) intravenously in anesthetized (pentobarbital or morphine ether) and atropinized dogs and cats were bradycardia, sustained fall of blood pressure (varying with dosage), absence of central vasomotor depression. Doses of 1 mg/kg. sometimes caused fleeting opposite cardiac and blood pressure effects. Subcutaneous, intramuscular and gastric administrations caused typical circulatory effects but the doses were higher. Accordingly, sparteine is a direct cardiac depressant in mammals as in amphibia. Electrocardiographically in unanesthetized dogs, the changes were typically quinidine-like. Typical antifibrillation was demonstrated mechanically and electrocardiographically in anesthetized dogs with 0.5–1.0 mg/kg. sparteine intravenously in auricular fibrillation produced by applying methacholine to the sinus node and slightly pinching the right auricle; higher doses (3–5 mg/kg.) were required to arrest or prevent electrical fibrillation. Other effects observed were: slight reduction in excitability of vagi and no antagonism of acetylcholine or methacholine (sparteine 5–10 mg/kg.); fleeting depression of phrenics; prompt respiratory failure, the cause of death after 30–50 mg/kg. intravenously (anesthetized dogs). Sparteine seems to be disposed of in the liver, corresponding circulatory effects requiring 3 to 5 times the dosage in the splenic vein compared with saphenous vein injection. Brodie's methyl orange method adapted to estimating sparteine in the blood (sensitivity 1 gamma/cc.) showed presence of the alkaloid for 2½ hours in the blood of dogs receiving 20 mg/kg. intravenously, thus suggesting a comparatively slow escape from the circulation.

Color and crystal tests for the identification of methadon and isomethadon. G. H. W. Lucas. *Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada.* Special reagents for the rapid colorimetric identification of methadon as the free base and as salts, and of isomethadon, have been prepared by combining 90% sulphuric acid solutions of some heavy metals (copper sulphite 1%, gold chloride 1%, palladium oxide 0.3%) and an 0.5% solution of ammonium vanadate in concentrated sulphuric acid. These metal solutions, which keep well, are combined in proportions of 8 to 1 with ammonium vanadate solution, which also keep well; the test solutions are useful for several hours only. A useful solution which will keep about a day may be made by mixing the ammonium vanadate solution and Marquis reagent in the ratio of 1 to 2. Powdered ferric sulphate added to the ammonium vanadate solution, when freshly made, is a useful reagent. The maximum sensitivity is reached with the gold and palladium reagents as one μg. of methadon may be detected. Many other salts of alkaloids, glycosides, barbiturates, antipyretics and antihistamine substances have been studied to determine any interference. A reagent containing 1 gm. of $HAuCl_43H_2O$ and 9.4 cc. of 55–58% HI solution appears to be the most sensitive for giving characteristic crystals with methadon (1:16,000). A 5% mercuric chloride gives very good and characteristic crystals but not so sensitive (1:4,000).

Local anesthetic action of some thiazolidone derivatives. F. P. Luduena and James O. Hoppe (by invitation). *Biology Div., Sterling-Winthrop Research Inst., Rensselaer, N. Y.* The local anesthetic activity of several substituted 2-phenyl-(3-alkylamino or 3-dialkylaminoalkyl)-4-thiazolidone compounds has been investigated by one or more of the following tests: sciatic nerve block (guinea pig), infiltration around the external canthus (rabbit), corneal instillation (rabbit), intradermal wheal (man) and spinal anesthesia (rabbit). With the diethylaminoethyl side chain the 3,4-methyl-enedioxyphenyl substituted compound (WIN 2125) was the most active (external canthus test) in comparison with analogs possessing the following phenyl substitutions: 4-methoxy,

3,4-dimethoxy, and 3,4,5-trimethoxy. WIN 2125 was as active and as toxic as procaine. The p-aminophenyl analog is inactive. In the alkylaminoalkyl series the activity is greater. Two of these compounds, WIN 2661 (2-(3,4-methylenedioxyphenyl)-3-isobutylaminopropyl-4-thiazolidone) and WIN 2663 (the cyclohexylaminoethyl analog) have been studied more extensively. These two compounds are 4–5 times more active than procaine in producing nerve block and spinal anesthesia. Intradermally in man they are more than 5 times more active than procaine. Topically, on the rabbit cornea, they are less active than cocaine. Tissue irritation of these compounds is low; intradermally in rabbits, WIN 2661 and WIN 2663 in a 1% concentration produced mild and moderate irritation respectively (trypan blue test). No signs of irritation were observed in man after the intradermal injection of 0.5% solutions of these compounds. The respective values (in mg/kg.) of WIN 2661 and WIN 2663 are as follows: mice, intravenously—40 ± 1 and 30 ± 1; subcutaneously—320 ± 30 and 190 ± 10; guinea pigs, intravenously—20 ± 1 and 17.5 ± 1.

Thromboplastic properties of some antiluetic drugs. DAVID I. MACHT. *Dept. of Pharmacology, Labys. of Sinai Hospital, Baltimore, Md.* The writer has already called attention to the fact that certain drugs, widely employed in medical therapeutics possess thromboplastic properties which may precipitate thrombo-embolic accidents. Among those studied experimentally were: digitalis, mercurial diuretics and the antibiotics penicillin and streptomycin. The author wishes to report now a new group of drugs which in animal experiments were found to shorten coagulation time of whole blood as tested by the Lee and White method. These are the antiluetic drugs: arsphenamine, neo-arsphenamine, mapharsen, bismarsen, bismuth salicylate and sodium-bismuth-tartrate. All of these were studied on rabbits and cats, with and without general anesthesia, and administered either by intravenous or intramuscular injection. They all exhibited marked thromboplastic effects. A remarkable exception is Thiobismol, which exerts very little influence on coagulation time. The organic mercury compounds, mercurochrome, merodicein, and merthiolate on intravenous injection also hastened coagulation, and even small doses of $HgCl_2$ had the same effect. So did tryparsamide, and the widely employed new antisyphilitic, penicillin. The above properties of antiluetics should be borne in mind, in analyzing the causes of occasional grave reactions occurring in the course of antisyphilitic therapy.

Observations on several cyclohexyl- and phenyl-alkyl-amines. EDWARD MACKO (by invitation) and EDWIN J. FELLOWS. *Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Penna.* In the present studies, the pharmacological actions of 1-Cyclohexyl-2-aminopropane (S-10) and 1-Cyclohexyl-2-methylaminopropane (S-25) the N-methyl modification of S-10, have been compared with their corresponding phenyl analogues. Observations in rats, rabbits and guinea-pigs disclosed the fact that the cyclohexyl derivatives were decidedly less active central nervous system stimulants than their corresponding phenyl modifications. The cardiovascular action of the present compounds has been analyzed and their relative acute toxicity determined in three species of animals. Subacute toxicity studies also were carried out on the cyclohexyl derivatives.

Fate of pentothal in man. LESTER C. MARK (by invitation), PHILIP A. LIEF (by invitation), E. M. PAPPER (by invitation), E. A. ROVENSTINE, ELEONORE BERNSTEIN (by invitation), and BERNARD B. BRODIE. *Depts. of Anesthesia and Biochemistry, New York Univ. College of Medicine, and New York Univ. Research Service, Goldwater Memorial Hospital, New York City.* A method was devised for estimating pentothal in biological material. This method is specific in that it does not include metabolic products of the compound in the measurement. The physiological disposition of the drug was studied. Negligible amounts of pentothal are excreted unchanged indicating that it is almost entirely transformed in the body. The drug metabolizes more slowly (about 15% per hour) than hitherto thought. The rapid recovery from anesthesia after the administration of small doses represents a shift of pentothal from plasma to tissues, rather than a rapid breakdown of the drug. Pentothal is extensively localized in various tissues of the dog. The tissue reservoir of pentothal may account for the long-lasting anesthesia obtained after the termination of sustained administration. The plasma concentration of pentothal is a function of plasma *p*H. Decreasing the *p*H to 6.8 in dogs by carbon dioxide inhalation lowered the pentothal level by about 35%, while increasing the *p*H by $NaHCO_3$ infusion to 7.6 produced a less striking change in the opposite direction. These changes were reversed when the *p*H was restored to normal. Evidence was found in human subjects of an acute tolerance to pentothal. Subjects maintained at high plasma concentrations of the drug awakened at higher plasma levels than those maintained at low plasma concentrations. Two transformation products have been isolated from urine. One of these has been purified and tested for anesthetic activity in mice. It has at most a mild sedative action.

Synthetic curare compounds. IV. N-Methylberbamine iodide and N-methyl-isotetrandine iodide. DAVID FIELDING MARSH and DONALD A. HERRING (by invitation). *Dept. of Pharmacology, West Virginia Univ. School of Medicine, Morgan-*

town, W. Va. Berbamine was isolated from *Berberis vulgaris*. The curariform activity of its derivatives, N-methyl-berbamine iodide (MB), and N-methyl-isotetrandine iodide (N-methyl-O-methyl-berbamine iodide or MI), was determined in comparison with the corresponding isomers, d-tubocurarine chloride pentahydrate (dT), and d-O-methyltubocurarine iodide trihydrate (MeOdT). Structurally the berbamine derivatives

	dT	MeOdT	MB	MI
	mg/kg. of body weight			
Albino rats				
LD_{50}	0.27	0.032	0.40	0.77
Rabbits				
Head drop 50	0.125	0.016	0.125	0.28
Holaday head drop	0.15	0.02	0.15	0.35
Cat gastrocnemius muscle				
Equivalent paralysis	0.125	0.02	0.125	0.30
Dog head drop (3')	0.16	0.02	0.17	0.48
Man head drop (3')	0.15	0.03	0.18	

differ from the d-tubocurarine compounds in that the tetrahydroisoquinoline rings are adjacent instead of being separated by the oxybenzyl groups. Although the berbamine derivatives are not as potent as the d-tubocurarine compounds, the lack of even the minor undesirable side effects possessed by d-tubocurarine make their clinical investigation seem reasonable.

Further data on the feeding of beryllium salts to laboratory animals. E. A. MAYNARD (by invitation), W. L. DOWNS (by invitation), J. K. SCOTT (by invitation) and H. C. HODGE. *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* Data obtained from larger groups of rats (10 males and 10 females) fed beryllium sulfate and beryllium carbonate at a dietary level of 5% confirmed data from small groups (2 males and 2 females) previously reported; i.e., complete inhibition of growth, some mortality, and a rickets-like condition which was accompanied by a bony-plate formation near the head of the tibia. Return to a stock diet with no beryllium sulfate brought about healing of the rickets but the bony plate remained. Pathological studies disclosed injury to kidney, liver, gonads and bone by 5% $BeSO_4$; rachitic-like bone injury to all rats and liver injury to some rats by 5% beryllium carbonate. Some bone deposition of beryllium was demonstrated by spectrochemical analysis. In the diet of rats, 10% beryllium sulfate caused 100% mortality in 10 days. When fed to dogs, 1 gm/kg/day of beryllium sulfate and 5 gm/kg/day of beryllium oxides had no effect on growth and produced no pathological changes.

'Curare-like' action of synthetic quaternary derivatives of stilbene. BERNARD P. MCNAMARA and TERESA SILVERMAN (by invitation). *Medical Div., Army Chemical Center, and Univ. of Maryland, School of Pharmacy, Baltimore, Md.* Four synthetic quaternary derivatives of stilbene have been investigated for their effects on neuromuscular transmission. The compounds are *1*) Stilbestryl bis (2-(4-morpholino-ethyl) ether, bis methiodide; *2*) Stilbestryl bis (2-diethylamino-ethyl) ether, bis methiodide; *3*) Stilbestryl bis (3-diethylaminopropyl) ether, bis methiodide; *4*) Stilbestryl bis (3-(2-methyl-piperidino) propyl) ether, methiodide. All four compounds exert a 'curare-like' action in rabbits and frogs. The paralyzing dose in rabbits is 0.1 mg/kg. or less for these ethers. Several differences between their actions and those of curare (Intocostrin) have been observed. Following intravenous administration of the stilbene derivatives in rabbits there is a delay of 5–15 minutes in the onset of paralysis. Neither prostigmine nor physostigmine antagonizes the paralysis produced in rabbits by these compounds.

Acute and subacute toxicity of 1,2,3 trichloropropane in mice and rabbits. WM. A. MCOMIE and T. R. BARNES (introduced by Hamiltion H. Anderson). *Div. of Pharmacology and Experimental Therapeutics, Univ. of California Medical School, San Francisco, Calif.* The approximate time required for 1,2,3 trichloropropane to kill (within 48 hours) 8 of 15 mice exposed to a vapor concentration of 30 mg/l., was 20 minutes (at 22°C.). Subsequently, liver damage accounted for 4 additional deaths, 7–10 days later. Immediate depression of respiration was noted, believed due to activity on the respiratory center. Subacute exposures over 10 days for 10 minutes each, at 15 mg/l., resulted in 7 deaths among 10 mice. In rabbits, 1,2,3 trichloropropane was an intense skin irritant, due in part to its solvent action on lipoidal components. Sloughing and cracking followed development of irritation and erythema. Ten applications of 2 ml. each over 100 sq. cm. during 15 days resulted in subdermal bleeding and a painful reaction. One of 7 animals died after repeated exposure. Congested lungs were apparent. The remaining rabbits survived and healed with return of hair growth within 6 weeks. Our findings with 1,2,3 trichloropropane confirmed previous reports on the toxicity of chlorinated hydrocarbons. Since 10-minute repeated exposures to saturated vapor-air mixtures caused delayed deaths in mice, prolonged observation after contact is indicated. Inhalation should be avoided, as well as skin exposure, since systemic pathologic changes may follow. Romensky (1872) provided experimental observations on response to 1,2,3 trichloropropane. Paralysis was observed in frogs. Sleep and lowering of body temperature were noted in rabbits. In man, 3 gm. orally caused sleepiness, marked headache, unsteady gait, and pain in the lumbar regions. Meyer (1899) reported paralysis of the respiratory center and serious effects on the heart.

A new antiadrenergic agent, 2-[N-p'-tolyl-N-(m' - hydroxyphenyl) - aminomethyl] - Imidazo-

line. HCl (C-7337). ROLF MEIER (by invitation) and FREDERICK F. YONKMAN. *Research Depts. of Ciba, Basle, Switzerland, and Summit, N. J.* This new antiadrenergic agent was developed by Marxer and Miescher in the imidazoline series as reported by Meier (N. Y. Acad. of Sciences Conference, 1947). It is adrenolytic in small doses and sympatholytic in higher concentrations. Its antiadrenergic properties have been demonstrated as follows: Protection of mice from lethal effects of epinephrine intravenously, reversalor in version of epinephrine hypertension in several species, inhibition or nullification of sustained vasoconstriction by epinephrine perfusion in the rabbit, obliteration of epinephrine's contractile effect of the isolated uterus of the rabbit, inhibition of the contractile action of epinephrine on the seminal vesicle of the guinea pig, nullification of adrenergic salivation in the cat and inhibition of adrenergic retraction of the feline nictitating membrane. The antisympathetic activity of C-7337 seems to be greater than that of Priscol and Dibenamine. Its general tolerability locally and systemically is of real potential advantage and in this sense resembles Priscol.

Comparative effect of digitoxin and ouabain on auriculo-ventricular conduction in the dog heart. RAFAEL MÉNDEZ and JOSE PISANTY (by invitation). *Dept. of Physiology and Pharmacology, National Inst. of Cardiology, Mexico, D. F., Mexico.* There has been considerable controversy as to whether differences, other than in speed of action or elimination, exist between the effects of different cardiac glycosides. The action of digitoxin (Digitaline Nativelle) and ouabain (Arnaud) upon A-V conduction was studied in dogs with the heart exposed under chloralose anesthesia. Electrodes were attached to the right auricle and the ventricles. Measurements of A-V conduction were taken during stimulation of the auricle at a constant frequency. The glycosides were injected at 30-minute intervals in doses calculated to kill the heart in about 3 hours. The results are summarized in the table.

Digitoxin vs. Ouabain on A-V Conduction

% of Lethal Dose (% of time necessary to kill the heart)	A-V Conduction Rate in % of Normal	
	Digitoxin (9 experiments)	Ouabain (9 experiments)
10	98.1	100.8
20	95.3	101.0
30	89.8	99.9
40	81.0	95.9
50	64.5	87.9
60 (Block beyond this dose)	56.8	77.0

Within its therapeutic range (30–50% of LD) digitoxin causes a significantly more pronounced lengthening of the A-V interval than ouabain. This effect although apparently mediated by the vagi seems specific on conduction for no significant differences were found in the effects of the two glycosides upon the sinus rate or rhythm. The effect is also independent of any action on the pacemaker as measurements were done under constant frequency stimulation.

Potency and pharmacological actions of neriifolin, a glycoside of *Thevetia Nerifolia*. KALMAN C. MEZEY (introduced by M. B. Chenoweth) *Cesar Uribe-Piedrahita Research Labys., Bogota, Colombia.* Neriifolin obtained from thevetia trees indigenous to Colombia has a cat unit of 0.20 ± 0.002 mg/kg. The minimal emetic dose in pigeons is 0.050 mg/kg. and in cats, 0.98 mg/kg. The minimal systolic dose in *Hyla labialis* by the U.S.P. XI frog method is 0.0035 mg/gm. Neriifolin is partially absorbed from the gastro-intestinal tract and exerts unusually marked action on isolated intestine. It is completely eliminated in about 72 hours. Except for oral absorption neriifolin most closely resembles ouabain pharmacologically.

Reaction of cholinesterase with diisopropyl fluorophosphate containing radioactive phosphorus. HARRY O. MICHEL (by invitation) and STEPHEN KROP. *Biochemistry and Pharmacology Sections, Medical Div., Army Chemical Center, Md.* The possibility of compound formation between cholinesterase and diisopropyl fluorophosphate (DFP) was investigated by reacting a cholinesterase preparation from the electric tissue of *Electrophorus electricus* with P^{32} labeled DFP. The preparation had an enzymatic activity of 2.6 gm. of acetylcholine hydrolyzed/hr/mg. of protein, as determined in 0.015M acetylcholine, 0.0342M $MgCl_2$ and 0.025M $NaHCO_3$ at *p*H 7.4 and 38°C. The radioactivity of the labeled DFP was 19 millicuries per gram. The reaction between the enzyme and DFP was permitted to proceed for 4 to 24 hours. The cholinesterase was then precipitated with 5% trichloroacetic acid (CCl_3COOH). Horse serum protein was added as a carrier. The precipitate was washed with CCl_3COOH and ethanol and then suspended in 0.02N NaOH. Aliquots were dried and counted directly. Phosphorus derived from DFP was found bound to protein in amounts directly proportional to the degree of inhibition of cholinesterase. The amount of phosphorus bound to the completely inhibited enzyme was equivalent to 0.100 μg. of DFP/mg. of cholinesterase. When DFP was replaced by sodium diisopropyl phosphate containing P^{32}, no phosphorus was bound by the enzyme.

Relative potencies of various cinchona alkaloids in patients with auricular fibrillation. WALTER MODELL, SAMUEL J. SHANE (by invitation) CONRADO DAYRIT (by invitation) and HARRY GOLD. *Dept. of Pharmacology, Cornell Univ. Medical College, Cardiovascular Research Unit of th*

Beth Israel Hospital, and Cardiac Service of the Hospital for Joint Diseases, New York City. Six alkaloids, quinine, natural quinidine, synthetic quinidine, dihydroquinidine, cinchonine and cinchonidine were compared in 18 patients with auricular fibrillation by means of their effect on the rate of the circus movement as revealed by the deflections in the electrocardiogram. After a control tracing a dose was given orally on an empty stomach, and tracings were taken at intervals of 2 hours to determine the maximum effect. This was repeated at intervals of several days with a series of doses from 0.1 to 2.0 gm. The percentage slowing was plotted against the log of the dose. The results of the 244 experiments show that natural quinidine (a mixture of quinidine and dihydroquinidine), synthetic quinidine, and dihydroquinidine are of equal potency, that quinine and cinchonidine are about one half as potent, and that cinchonine is the least active, about one third as potent. The discrepancy between these results and those stated in the literature may be explained by the fact that the latter were not based on dosage-response curves and the doses used were so large as to fall in the insensitive range of dosage-response.

A method for the estimation of chloroform in small quantities of blood. LUCIEN E. MORRIS and EVAN L. FREDERICKSON (introduced by O. Sidney Orth). *Depts. of Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* An improved method is presented which utilizes ether extractions and the Fujiwara color reaction of alkaline pyridine for rapid, accurate, photoelectric determination of chloroform in 1 cc. quantities of blood. Estimations of chloroform in the blood of anesthetized dogs gave values of from 18 to 50 mg. %, which compares closely with values obtained by earlier workers using more laborious or less delicate methods. Values found during clinical anesthesia indicate a marked species variation. Estimations of chloroform in the blood of 50 patients during anesthesia for major surgical procedures with chloroform as the sole agent gave an average value of 10 mg. % during maintenance.

Effect of pentobarbital on response of the cardiovascular system of dogs to epinephrine, acetylcholine and tilting. JAMES L. MORRISON, HARRY A. WALKER and ARTHUR P. RICHARDSON. *Dept. of Pharmacology, Emory Univ. School of Medicine, Emory Univ., Ga.* It has long been recognized that anesthesia alters the response of the circulation to the administration of many drugs; however, a quantitative description of these changes is at present lacking. We have attempted to obtain this information for pentobarbital. Unanesthetized dogs were tied to a tilting operating table and prepared for recording of blood pressure and intravenous injection of drugs. The response of the circulation to the injection of a standard dose of epinephrine and acetylcholine in the unanesthetized state was determined. In addition, the response of the circulation to a sudden change in position was determined by tilting the animal from a horizontal to vertical position. Following these procedures, graded doses of pentobarbital were administered and the response to the standard test procedures redetermined after each dose of anesthetic. The injections of pentobarbital were continued until death of the animal. In a series of 6 dogs, the average lethal dose was 55 mg/kg. Gradually increasing doses of pentobarbital produced the following results: *a*) In the resting state pentobarbital produced a gradual fall in blood pressure with a decrease in pulse pressure and a marked increase in pulse rate. *b*) There was an increase in sensitivity to epinephrine characterized by a progressively greater rise in blood pressure, accompanied by a decrease and finally abolition of reflex bradycardia. *c*) There was an increase in sensitivity to acetylcholine characterized by a more prolonged fall in blood pressure and accompanied by a progressive decrease and final loss of reflex tachycardia. *d*) Pentobarbital produced a moderate postural hypotension accompanied by a loss of reflex tachycardia on tilting. These results can best be explained as being due to a selective central depression of reflex control of the circulation.

Antihistaminic properties of the β-haloalkylamine (dibenamine) series of adrenergic blocking agents. MARK NICKERSON and FRANCES B. HARRIS (by invitation). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah.* The antihistaminic activities of 75 congeners of Dibenamine were determined by testing their ability to prevent histamine-induced contraction of the guinea pig ileum. All drugs were also tested against methacholine on the same preparation; with a very few exceptions the antihistaminic properties were found to be independent of significant cholinergic blocking activity. Antihistaminic activity in this series is not parallel to adrenergic blocking activity, but no compounds devoid of adrenergic blocking activity are among the more active antihistaminics. The β-haloalkyl grouping is essential for high activity. Substitution of a β-dimethylaminoethyl radical which provides maximal activity in several series of antihistaminics leads to inactivation. Phenoxyethyl (particularly 2-substituted) and α-naphthylmethyl amines are the most active. Unsymmetrical N-phenoxy-ethyl-N-benzyl compounds are almost always more active than N-diphenoxy-ethyl or N-phenoxyethyl-N-ethyl congeners. Several compounds with activities more than 10 times that of Benadryl are included in the series. In contrast to Benadryl and Pyribenzamine which produce a readily reversible 'equilibrium' blockade, members of this series produce a very

persistent blockade similar to that observed in connection with their adrenergic blocking action. Development of this blockade appears to occur in two stages. During the first stage these agents exhibit a competitive equilibrium with histamine which is no longer present after completion of the blocking action. A competitive inhibition of the action of these agents by Benadryl indicates a common site of action.

Mechanism of the acute lethal action of epinephrine in rats. MARK NICKERSON, JOHN BERGHOUT (by invitation) and ROBERT N. HAMMERSTROM (by invitation). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah.* Protection against the lethal effect of epinephrine in rats and mice has recently been employed as a test for adrenergic blocking activity. However, lack of information regarding the mechanism of death allows no definite conclusions regarding the specificity of this test. Sprague-Dawley rats were injected intraperitoneally with lethal doses of epinephrine, and protection was then provided by various physical procedures. Pentobarbital and heparin, employed in some of the tests, were found not to alter significantly either mortality or 'epinephrine' content of heart muscle as determined colorimetrically. Artificial respiration provides complete protection against several multiples of the lethal dose of epinephrine, but does not alter the 'epinephrine' content of the myocardium, which may be several times as great as that of control animals dying from smaller doses. Systemic arterial pressure stabilization also provides marked protection. In none of the animals was significant pulmonary edema observed. It is concluded that the lethal effect of epinephrine in rats is directly related to the induced increase in systemic arterial pressure which in turn induces respiratory failure. The mechanism of the last step is not clear, but it is not the same as that involved in transient 'epinephrine apnea'. Dibenamine protects against the lethal effects of epinephrine but does not prevent 'epinephrine apnea'. Epinephrine toxicity in rats appears to be unrelated to an accumulation of the agent (or related catechols) in the myocardium.

Timed intravenous infusion of metrazol or strychnine in anticonvulsant testing. MARSHALL J. ORLOFF (by invitation), H. L. WILLIAMS (by invitation), and CARL C. PFEIFFER. *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, Ill.* The timed intravenous infusion of metrazol (0.5%) and strychnine (0.01%) makes possible an accurate graded evaluation of anticonvulsant compounds. The solutions are injected at the rate of 0.05 cc. every 10 seconds into the tail vein of the mouse. Three signs of reaction follow each other in sequence as the concentration of metrazol is increased in the blood. These have been designated as the 'first twitch,' the 'pseudoconvulsion,' and the 'persistent convulsion'. 'Tridione', phenobarbital, 'Dilantin', 'Phenurone', and 'Myanesin' have been used as anticonvulsants to test the accuracy of the method and to compare it with the subcutaneous metrazol method in general use. The chief advantages of the method are: *1*) fewer animals are necessary in order to evaluate the anticonvulsant; *2*) it is less time-consuming than the subcutaneous metrazol method; and *3*) it exhibits a high degree of sensitivity.

Effects of excess carbon dioxide in clinical anesthetic atmospheres upon cardiac automaticity and liver function. O. SIDNEY ORTH, RALPH M. WATERS, LUCIEN E. MORRIS (by invitation), J. L. SIMS (by invitation), and ROBERT T. CAPPS (by invitation). *Depts. of Pharmacology, Anesthesiology and Internal Medicine, Univ. of Wisconsin Medical School, Madison, Wis.* In the administration of anesthetics clinically there frequently is an accumulation of carbon dioxide in the respired atmosphere and a resulting systemic hypercarbia occurs. To determine the effects which such a condition might produce on cardiac automaticity and hepatic function in the human subject, moderate accumulations of carbon dioxide were produced either by permitting definite rebreathing or by the continuous addition of small amounts of carbon dioxide to the anesthesia bag while chloroform or ether was used as the sole anesthetic agent. Electrocardiographic observations were made continuously with the use of a direct recording machine and tracings were taken whenever desired. Analysis of the respired atmosphere was made every 3–5 minutes to prove the continuous presence of oxygen at or usually above 30%, and to know the percentage of carbon dioxide in the mixture. Blood samples were obtained periodically for determinations of hemoglobin, oxygen and carbon dioxide content and carbon dioxide capacity. Five tests of liver function, which included icterus index, cephalin-cholesterol flocculation, prothrombin time, bromsulfalein retention, and thymol turbidity, were made before the operation, 24 hours postoperatively, and every second day thereafter for 4 times or until liver function tests returned to normal.

Influence of roentgen rays on potency of penicillin. MARCUS OSTRO (by invitation) and DAVID I. MACHT. *Depts. of Pharmacology and Radiology, Sinai Hospital, Baltimore, Md.* Aqueous solutions both of crystalline penicillin (C.S.C.) and amorphous penicillin were treated with x-rays in open glass beakers, and compared pharmacologically with control non-radiated solutions of the same preparations. The apparatus employed was a water-cooled therapy tube operated on 200 K.V. and 20 M.A. with a target distance of 50 cm. The rays

employed were passed through a filter of 1 mm. aluminium and 2 mm. copper in some experiments, and a filter of 1 mm. aluminium and 0.5 mm. copper in others; the dosage employed in each case being the same. Dosages ranging from 93r to 280r were given to the solutions. Three kinds of tests were used for pharmacological comparison. *1*) Phytotoxic tests on root growth of lupinus seedlings in plant physiological solutions revealed a greater phytotoxicity after irradiation through the 2 mm. Cu filter, ranging from 20 to 30%. *2*) The thromboplastic properties of penicillin after irradiation were also markedly increased, often 50% or more. *3*) Bacteriological tests were made through the cooperation of Drs. Lawrence and Jerome Martin of the C.S.C. Research Laboratories. These revealed no difference in chemotherapeutic and bacteriostatic potencies between x-rayed and control solutions. The difference in the phytotoxic and thromboplastic properties occurred only when solutions of penicillin were irradiated, but did not occur when solid penicillin was exposed to x-rays. Irradiations of mice with above filtered x-rays in doses from 63r to 93r did not render the animals more vulnerable to subsequent injections of large doses of normal penicillin.

Experimental safety of sorbitan monostearate and its polyoxyethylene derivatives. SIDNEY G. PAGE, JR. (introduced by HARVEY B. HAAG). *Medical College of Virginia, Richmond, Va.* Three products were submitted for investigation, containing Sorbitan Monostearate (Span 60) and Polyoxyethylene Sorbitan Monostearates (Tween 60 and Tween 61). Weighed contents were suspended in chocolate syrup and administered 3 times daily for 28 days to a group of 60 subjects. These ranged in age from 20 to 49 and were physicians, technicians or junior medical students. Physical examinations, blood and urine studies were made before administration and after 14 and 28 days of continuous administration. Further examinations were made 14 days after discontinuing administration of these products. The clinical observations included body weight, blood pressure and pulse measurements; the urinalyses included color, reaction, specific gravity, sugar and albumin, in addition to a microscopic examination. The blood examinations included determinations of hemoglobin, erythrocytes, leukocytes and differential examination; chemical determinations were made of the plasma protein, NPN, sugar, calcium and cholesterol. No significant variations were observed in any of the physical, microscopic or other examinations during this entire study. These substances did not produce any untoward effects in this investigation.

Effects of inhalation of high concentrations of carbon dioxide. THOMAS M. PARRY (by invitation), JOSEPH N. SPENCER (by invitation), WILLIAM B. DRAPER, RICHARD W. WHITEHEAD and ROBERT L. ARENDS (by invitation). *Dept. of Physiology and Pharmacology, Univ. of Colorado Medical Center, Denver, Colo.* Observations on dogs in diffusion respiration indicate that there is a progressive rise in blood carbon dioxide and a concomitant fall in blood *p*H during respiratory arrest. To determine the effect of the concentrations of CO_2 upon the blood *p*H, dogs were administered 10, 20, 30 and 40 % of carbon dioxide in oxygen for a period of 45 minutes while under light thiopental anesthesia. Changes in heart rate, E.C.G., and blood *p*H were noted, as well as the respiratory, anesthetic and toxic effects of the carbon dioxide mixtures. The inhalation of the carbon dioxide mixtures produced no significant change in the heart rate. Likewise, the only alteration in the E.C.G. was an increase in the amplitude of the T wave, which returned to normal with the return of the blood to normal. The venous blood *p*H fell rapidly during the first 15 minutes of carbon dioxide inhalation, but thereafter decreased only slightly. The *p*H fell from a control average of 7.42 to 7.24, 7.05, 6.83 and 6.79 during inhalation of 10, 20, 30 and 40% carbon dioxide, respectively. Upon removal of the animals from the carbon dioxide atmosphere the *p*H rose rapidly. With 30 and 40% carbon dioxide the *p*H rose to 7.28 within 30 minutes while with 10 and 20% carbon dioxide it had returned to normal.

Effects of pituitrin, pitressin and pitocin on blood pressure and cardiac automaticity as modified by various anesthetic agents. CARLOS E. PARSLOE (by invitation), LUCIEN E. MORRIS (by invitation) and O. SIDNEY ORTH. *Depts. of Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* Accidents which have occurred following the administration of pituitary preparations to anesthetized patients make it desirable to reevaluate the pharmacological action and therapeutic uses of these drugs and to study any possible incompatibility which they may have with various anesthetic agents. Eighty-five experiments were made with 26 dogs. Anesthetic agents employed were either cyclopropane, ether, chloroform, nembutal, chloretone or procaine. Unanesthetized animals also were studied. Blood pressure determinations and continuous electrocardiographic records were made and upon analysis subsequent to intravenous injection of each of the pituitary fractions the results were found to confirm and extend previous observations. Blood pressure response showed marked variation dependent on the type of anesthetic used. Most striking was a characteristic, almost precipitous fall in pressure observed after either pituitrin or pitressin during cyclopropane anesthesia. There were no significant changes following the use of pitocin. Alterations of cardiac rhythm were ob-

served in each of 14 conscious animals and in 9 with spinal analgesia. One-fifth of the animals tested with pituitrin or pitressin during cyclopropane anesthesia exhibited some type of arrhythmiae which was thought to be correlated with injection of the drug and associated with coronary constriction and a resultant myocardial hypoxia predisposing to cardiac dysfunction in the presence of anesthesia. Side effects of pituitrin administration, such as modification of respiratory activity to r duce effective exchange or retching and vomiting which may mechanically obstruct respiration, lead to increased hypoxia and are additional factors increasing the hazard of accident. Clinical electrocardiographic studies are in progress.

Antiseptic properties of certain mercurial diuretics. S. ANDERSON PEOPLES and ANN MCCORMICK (by invitation). *Division of Veterinary Science, Univ. of California, Davis, Calif.* Mercurial diuretics are rapidly excreted in the urine after intravenous administration, and if the compounds should then break down to give a sufficient concentration of ionized mercury, they would act as urinary antiseptics. In order to test this possibility, the in vitro antibacterial action of Salyrgan (I), Mercuzanthin, (II) Mercuhydrin (III) and mercuric chloride were tested against *Streptococcus faecalis, Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus,* and *Corynebacterium renalis.* The concentration of mercury furnished by the compound in micrograms/cc. required to inhibit growth was determined turbidometrically and the bactericidal concentration by subculture in thioglycollate media. The apparent percentage of the mercury in the compound which is ionized can be calculated from the ratio of the mercury as mercuric chloride to that of the compound required to produce the same endpoint. II was the most active, inhibiting all the organisms at 3–12, indicating that 50% of the mercury was ionized. I and III caused inhibition at 6–200, indicating 3–24% ionization. The bactericidal concentrations were difficult to determine accurately and varied from 12–400. These results were not materially altered when blood serum was added to the media nor when urine of *p*H 6.7 was used as media. The activity of all the compounds was increased by changing the *p*H of the media to either 5.0 or 8.0. These bacteriostatic and bactericidal concentrations can easily be reached in the intact animal and tests of these compounds in experimental urinary infections are now underway.

Release of various proteins from incubated surviving liver slices. RITA L. PESSOTTI (by invitation) and RALPH W. BRAUER. *Dept. of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans, La.* In an effort to elucidate the factors which control the distribution of various plasma proteins between the hepatic cells and the blood plasma, liver slices from dogs and rats have been incubated in protein-free or protein-containing solutions under various conditions. Analytical procedures applied include total protein determinations, esterase activity determinations, cholinesterase activity determinations, and electrophoretic analysis at *p*H 7.8. Results indicate that the release of protein with respect to the quantity, quality, and time course does not differ markedly as between different lobes of a liver obtained from the same animal; on the other hand, wide differences are obtained in going from one animal to the next. On incubation with Krebs solution at *p*H 7.4, the release of cholinesterase activity and of 'alpha globulins' procedes much more rapidly than that of total protein. The addition of bovine plasma albumin to the incubation solutions accelerates and increases the amount of cholinesterase activity and of 'alpha globulins' released. These results parallel observations previously made in intact dogs under conditions of plasmapheresis or albumin transfusions. If liver slices are repeatedly transferred to fresh samples of Krebs solution a reduction in the rate of release of protein and of enzyme activity is usually observed after 2 or 3 transfers under the conditions of these experiments.

Nephrotoxic action of a cyanine dye, 1′-ethyl-3,6 - dimethyl - 2 - phenyl - 4 - pyrimido - 2 - cyanine chloride. LAWRENCE PETERS and AEME HIGASHI (by invitation). *Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.* Distribution studies in dogs following I.V. administration of dye showed its concentration in the kidneys to be higher than that in other organs. However, recovery from the urine never exceeded 10% of the dose given. Microscopic examination of unstained frozen-dried sections revealed the presence of large amounts of dye in the cells of the convoluted tubules. Doses greater than 10 mg/kg. had a pronounced effect on renal function. Transient albuminuria occurred. Nitrogen retention, reflected in markedly elevated BUN and plasma creatinine concentrations, usually reached a peak between 48 and 96 hours, returning to normal over a variable period, depending on magnitude of dosage and individual susceptibility. Renal clearance values for inulin, creatinine, PAH (low and high plasma levels), glucose and sulfamerazine were all depressed during azotemia. The clearance of PAH (C_{PAH}) at low plasma levels (0.8–1.8 mg. %) fell less than the inulin clearance; hence the filtration fraction (C_I/C_{PAH}) was decreased. During recovery the clearances returned to levels equal to or only slightly in excess of preinjection values. CPAH was again exceptional in that it increased 200–300%, resulting in a filtration fraction ½ to ⅓ of that existing before dye infection.

Though the dye possessed vasodepressor properties, the effects were obtainable following dye infusion at rates producing little or no fall in femoral arterial pressure. The animals were killed for post-mortem examination. Microscopically, sections revealed nephrotic changes confined principally to the cytoplasm of the tubule cells. Nuclear changes existed in some cases to be discussed.

Actions of 2,6-diaminopurine in mice, rats, and dogs. FREDERICK S. PHILIPS and J. B. THIERSCH (by invitation). *Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.* Recent reports finding 2,6-diaminopurine; *1*) an antagonist of folic acid and purine metabolism in microorganisms (Hitchings, et al. *J. Biol. Chem.* 174: 765, 1948); *2*) a precursor in the synthesis of guanine in rats (Bendich and Brown, *J. Biol. Chem.* 176: 1471, 1948); and *3*) therapeutically active against leukemia in mice (Burchenal, et al. *Cancer*, in press) led to studies of its pharmacology. Its toxicity by parenteral administration was determined in mice, rats, and dogs. Acutely toxic doses were fatal within 48 hours and in dogs caused protracted emesis, hemorrhagic diarrhea, severe losses of fluid and electrolyte, hemoconcentration, and collapse. However, daily doses as great as $\frac{1}{8}$ to $\frac{1}{4}$ LD-50 were tolerated by rats and dogs for 1 to 2 weeks or longer. Signs of chronic intoxication included anorexia, progressive weight-loss, and in peripheral blood, reticulocytopenia, leucopenia, and moderate anemia. Bone marrow from rats sacrificed in terminal stages of chronic intoxication was semi-fluid exhibiting significant depletion of both myeloid and erythroid elements. In dogs receiving daily doses serial bone-marrow aspiration revealed progressive diminution of total nucleated cell counts involving greater decreases in erythroid than in myeloid cells. Terminally nucleated erythroid cells were almost absent while myeloid cells in all stages were present, but, in less than normal quantities. In its effects on marrow and intestinal epithelium 2,6-diaminopurine resembled 4-amino-pteroylglutamic acid with regard to site of action. However, the actions of the purine and the folic adid antagonist could be differentiated in so far as the former failed to elicit megaloblastic erythropoiesis in dogs while the latter appeared to be more uniformly toxic to both myelopoiesis and erythropoiesis.

Quantitative estimation of pyribenzamine in blood. ALBERT J. PLUMMER (introduced by GEORGE L. MAISON). *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.* With the cooperation of the Ciba Pharmaceutical Company, a chemical method for the quantitative estimation of pyribenzamine in blood has been developed. While this method is not as sensitive as the fluorimetric method of R. Mizzoni it is simpler. The method: 20 ml. of blood are added to 30 ml. of 15% Trichloracetic Acid. The precipitated protein is removed by filtration and the filtrate, about 35 ml., is rendered just alkaline with 10% sodium hydroxide. The filtrate is extracted with two 15 ml. portions of ether and these are combined and evaporated to dryness over a warm water bath. The residue is dissolved in .05 ml. of .01% sulfuric acid and 1 ml. of water. After allowing 5 minutes for solution the liquid is transferred to a Klett colorimeter tube and diluted to 5 ml. Iodine solution, 0.25 n, 0.04 ml. is added and after 10 minutes the turbidity is read in colorimeter using No. 50 filter. The amount of pyribenzamine may be estimated by comparison with the readings obtained by developing turbidity in solutions of pyribenzamine hydrochloride of known concentration. It has been possible to measure with an error of plus or minue 5% amounts as low as 0.05 mg. pyribenzamine hydrochloride added to 100 cc. of human blood. The method is also applicable to the determination of antistine in blood. Here however, at present, the lowest detected concentration has been 0.3 mg. per 100 ml. of blood. No property of normal blood has interfered with the application of the method.

Augmentor effect as postulated by the electromotive theory of drug action. HARRY J. PRATT (by invitation) and R. BEUTNER. *Hahnemann Medical College, Philadelphia, Pa.* According to the electromotive theory of drug action, the stimulating action of a drug is proportioned to the electromotive change which it brings about at a phase boundary of water and an 'oil'. If the stimulant drug is an alkaloid like pilocarpine, the electromotive effect on the phase boundary is a negative variation, like the negative variation brought about by stimulation of nerve. This electric effect of the drug is enhanced by addition of sodiumbenzoate or other sodium of organic acids, but only within certain limits; a larger or smaller addition of benzoate will decrease the electric effect, (Beutner, *J. Pharm.* 34: 29, 1928). The stimulating action of pilocarpine, as tested on the isolated intestine of rabbit, is likewise enhanced by addition of sodiumbenzoate; this also occurs only within certain limits of concentration as experiments show, but only two single measurements by Storm van Leeuwen and von György were available so far to test the theory. In order to obtain additional data, the following experiments were made. To a strip of rabbit duodenum, suspended in Tyrode solution, 0.03 mg. of pilocarpine was added. They produced a contraction of 45 mm. This was reduced 43% by 0.03 mg. of sodiumbenzoate; it was potentiated 30% by 0.165 mg., 71% by 0.33 mg., 20% by 1.65 mg., 33% by 3.3 mg., and again reduced 28% by 16.5 mg. or 70% by 33 mg. These data show that the potentiation of the constricting action of pilo-

carpine occurs only within certain limits as does the electric effect.

Inhibition of myocardial reactions to epinephrine and nor-epinephrine by nitroglycerine. W. RAAB and EUGENE LEPESCHKIN (introduced by F. J. SICHEL). *Division of Experimental Medicine, Univ. of Vermont, College of Medicine, Burlington, Vt.* In atropinized cats the blood pressure and electrocardiogram were recorded after intravenous injection of epinephrine and nor-epinephrine (probably identical with sympathin) and after faradic stimulation of the stellate ganglia. The injections were followed by elevation of the blood pressure, tachycardia and transient flattening or inversion of the T-wave in leads CR1 and CR4 (between the 15th and 50th seconds) and later (until about the 160th second) usually by elevation of T. The effects of epinephrine and nor-epinephrine were almost identical, and similar to those produced by stimulation of the stellate ganglia. If nitroglycerine was injected together with epinephrine or nor-epinephrine, the effect of the latter on blood pressure and tachycardia was weakened, the depression of the T-wave was either entirely abolished or markedly diminished. This effect was considerably greater than would correspond to the slight or moderate elevation of T, sometimes elicited by nitroglycerine alone. Since the depression of T had proven entirely independent of blood pressure and heart rate, the inhibiting effect of nitroglycerine is assumed to take place in the myocardium itself through interference with the metabolic action of the sympathomimetic amines. As epinephrine is believed to dilate the coronaries, it seems unlikely that the inhibition of the epinephrine-induced electrocardiographic changes through nitroglycerine should be due to a further coronary dilatation. The T-wave depression after stimulation of the cardiac sympathetic was likewise inhibited by nitroglycerine. It is probably due to discharges of nor-epinephrine into the myocardial cells.

Purification of the toxic principle of agene-treated gluten for pharmacological study. JACK L. RADOMSKI, GEOFFREY WOODARD, and HENRY N. FUYAT (introduced by ARNOLD J. LEHMAN). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* To facilitate the study of the pharmacology of canine hysteria it was desirable to purify the responsible etiological agent. The starting material was prepared by the hydrolysis of agene (NCl_3)-treated gluten with pancreatin. The *p*H of a batch of toxic hydrolysate was depressed stepwise to progressively lower values by the addition of a cationic exchange resin (Ion X) in its H^+ form. The resin was filtered off each time a desired *p*H was reached. This process was repeated until the *p*H was depressed beyond the range of interest. Thus, the peptides and amino acids of this hydrolysate were fractionated according to the *p*H range in which they become adsorbable. Each of the batches of resin was separately eluted with dilute ammonia and the toxicity of the eluate determined by oral administration to dogs. It was found that the toxic principle was adsorbed exclusively between *p*H 3.35 and *p*H 3.95. It is felt that this method is generally applicable for the separation of peptides from protein hydrolysates and constitutes an improvement on previously published techniques. Further concentration was effected by washing the resin with dilute hydrochloric acid before the toxic principle was eluted with ammonia. The eluate may be dried to a white water-soluble powder with an acute toxicity of 500 mg./kg. to dogs. This represents a 10-fold purification over the starting material. Still further purification was achieved by extraction with organic solvents. The pharmacological behavior of the partially purified toxic principle in dogs, monkeys, rabbits, and rats was investigated.

Metabolic factors contributing to the course of pentothal anesthesia. CLAUDIA M. RATLIFF (by invitation), A. H. MALONEY, JAMES R. TUREMAN (by invitation) and WALTER M. BOOKER. *Dept. of Pharmacology, School of Medicine, Howard Univ. Washington, D. C.* Previous work reported from this laboratory has called attention to hyperglycemia and glycogenolysis under prolonged pentothal anesthesia. An extension of that work pointed out the indication for insulin during pentothal anesthesia. In the present work experiments are reported in which specific units of insulin are administered at regular intervals (intramuscularly) or by constant drip (intravenously) sufficient to keep the blood sugar in normal range during three hours of pentothal anesthesia. It has been found that insulin-treated animals require greater quantities and more frequent administration of pentothal sodium to keep them in surgical anesthesia as compared with untreated controls. Insulin treated animals have slightly higher liver glycogen values than untreated animals at the end of 3 hours of anesthesia. There is some evidence that the glycogen storage at the liver influences the destruction of pentothal, since in animals on high carbohydrate and on high protein diets, where the liver glycogen is high, accumulation of pentothal at the liver from hour to hour is less than in animals on normal diets. Taking liver samples every hour for pentothal and micro glycogen determinations, it has been found that the more rapidly the glycogen falls, the greater is the build up of pentothal at the liver. Furthermore, in animals on normal diet the liver is efficient the first hour in destroying pentothal; but as anesthesia is pushed into the second and third hours more and more pentothal can be demonstrated at the liver.

Induction of idioventricular rhythms by 1-1-1 trichlorethane and epinephrine. BARBARA R. RENNICK (by invitation), S. DONALD MALTON (by invitation), G. K. MOE and M. H. SEEVERS. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* Methyl chloroform was recommended as an anesthetic agent 60 years ago, but has never had clinical trial. In this laboratory attempts to induce anesthesia in 2 dogs without premedication led to sudden death, presumably cardiac in origin, in both animals. In 5 dogs under barbital anesthesia epinephrine was injected after administration of repeated small doses of methyl chloroform. Ventricular extrasystoles and ventricular tachycardia were produced in all animals. Maximum sensitization of the heart to epinephrine occurred after the administration of 0.25 to 0.4 cc/kg. of methyl chloroform. Further administration of the agent raised the threshold dose of epinephrine, in part because of the resultant severe hypotension. Ventricular arrhythmias can be more regularly induced with methyl chloroform than with chloroform, but the sensitivity of the heart appears to be less than with cyclopropane.

Competitive inhibition of convulsions in guinea pigs. R. K. RICHARDS and K. E. KUETER (by invitation). *Dept. of Pharmacology, Abbott Research Laboratories, North Chicago, Ill.* The authors have shown that para aminobenzoic acid (PABA) and diethylaminoethanol (DEAE), the split products of procaine, can either singly or, more efficiently, as a mixture inhibit the convulsions caused by i.m. injection of procaine in guinea pigs when administered i.p. prior to this drug. This work has been extended by using the dibutylamino-propanol (DBAP) ester butyn, a more potent local anesthetic of the PABA series. 30 mg/kg. of butyn sulfate i.m. caused convulsions in 90% of the guinea pigs. This incidence could be reduced to about $\frac{1}{3}$ by pretreatment with 400 to 600 mg/kg. PABA. DBAP and similar long-chain amino alcohols were too toxic for testing their possible antagonistic effect against butyn as it was demonstrated with DEAE in the case of procaine. Metycaine was studied because it is an ester of benzoic acid rather than of PABA. 45 mg/kg. of metycaine HCl was convulsive in 82% of the animals. The incidence of convulsions was reduced to about 55% by premedication with comparable doses of either benzoic acid or PABA. As with butyn the metycaine side chain, 2-methylpiperidinopropanol, was relatively too toxic to investigate its possible antagonistic effect. These findings will be discussed from the viewpoint of the theory of competitive inhibition on the basis of structural similarity and the changes in pharmacologic properties which occur if the tertiary amino group of the amino alcohol is converted into a quaternary ammonium base which deprives the esters of the local anesthetic properties.

Action of caffeine and aminophylline as respiratory stimulants in man. GLENN H. RICHMOND (introduced by C. L. GEMMILL). *Dept. of Pharmacology, Univ. of Virginia, Medical School, Charlottesville, Va.* The respiratory stimulating effect of CO_2 was used as a functional test to study the mechanism of action of caffeine and aminophylline as respiratory stimulants. Ventilation minute volumes were obtained with the subject breathing atmospheric air, 3% CO_2 in O_2, and 5% CO_2 in O_2 before and after the administration of 0.25 gm. of caffeine subcutaneously and before and after the administration of 0.25 gm. of aminophylline subcutaneously. The average increase in ventilation minute volume, of 6 subjects, when breathing 3% CO_2 was found to be 23% greater after caffeine than before. When breathing 5% CO_2 the increase was 51.7% greater after caffeine than before. It was concluded from this study that caffeine acts on the respiratory center by increasing its sensitivity to CO_2. Aminphylline was found to have no significant effect on the response of respiration to CO_2.

Lethal and electrocardiographic changes produced by quinine dihydrochloride in monkeys infected with *P. knowlesi*. R. H. RIGDON. (introduced by CHAUNCEY D. LEAKE). *Laby. of Experimental Pathology and Heart Station Univ. of Texas, Medical Branch, Galveston, Texas.* During our studies on malaria, quinine dihydrochloride was given to 31 monkeys. Some of those with a severe infection died immediately following an intravenous injection of quinine. Since other animals of equal weight survived a similar dose, experiments were designed to study this variation. To determine the effect of quinine on the electrocardiogram, 20 monkeys were used, 9 of which were normal and 11 infected with *P. knowlesi*. Limb leads and an apical chest lead were obtained and immediately thereafter quinine dihydrochloride was given intravenously and a second group of tracings was made during an interval of 10 minutes. A series of tracings were made on 4 monkeys given quinine both before and after their infection. In some experiments the dosage of quinine was varied to study its effect, while in other experiments the dose was kept constant to determine the variations accompanying the changes in the degree of parasitemia and the anemia. The lethal doses of quinine were 56 and 77 mg/kg. for two normal monkeys. In contrast, the following doses, 28, 28, 28, 31, 37.5 and 47 mg/kg., were lethal for malarial infected monkeys who had red blood cell counts between 1.9 and 3.24 million. Malarial infection, per se, although severe, did not appear to affect the electrocardiogram in the majority of cases. In 3 monkeys the deleterious conduction and other electrocardiographic effects of quinine

were found to be greater when infected than when normal. In malarial infected monkeys the electrocardiographic changes following quinine appeared to parallel the degree of anemia rather than the degree of parasitemia.

Renal clearance of iodide in the dog. DOUGLAS SHEPARD RIGGS. *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.* Simultaneous clearances of iodide, chloride and creatinine have been determined in dogs maintained on a low-chloride, high-protein diet. The average glomerular filtration rate measured by the creatinine clearance was about 100 cc/minute. When plasma iodide concentrations were 1.6 mEq/l. or less, the clearance of iodide was remarkably low, ranging from 0.05 to 0.79 cc/minute. With higher plasma iodide levels the clearance increased to as much as 8 cc/minute at 11 mEq/l. of plasma. When plasma iodide was maintained at about 0.4 mEq/l. and chloride excretion was elevated by the oral administration of sodium chloride, the clearance of iodide increased markedly to as much as 17.7 cc/minute. The iodide clearance was always somewhat greater than the chloride clearance, the difference being most marked when the chloride excretion was moderate. When chloride excretion was very high, the kidney seemed less able to differentiate between the two halides. This dependence of iodide excretion on chloride excretion resulted in prolonged retention of iodide in dogs whose kidneys were conserving salt. For example, a plasma iodide level of 1.2 mEq/l. was maintained without appreciable change for 24 hours after a single subcutaneous injection of sodium iodide in a fasting animal. Administration of sodium chloride occasioned a prompt and profound fall in plasma iodide. These experiments indicate that the tubular reabsorption of iodide in the dog may be almost complete when total halide excretion is minimal.

Effects of 3-acetoxy phenoxy trimethylammonium methylsulphate on neuromuscular function. WALTER F. RIKER and W. CLARKE WESCOE (by invitation). *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* In the gastrocnemius-soleus preparation of the intact cat the close intra-arterial injection of 3-acetoxy phenoxy trimethylammonium methyl-sulphate (Nu 2017, Hoffmann-LaRoche, Inc.) produces an immediate maximal contraction of the muscles followed by intense local fasciculations. The fasciculations disappear completely within one minute. If the dose is sufficiently large, the fasciculations become generalized. The close intra-arterial injection of 5 μg/kg. potentiates the response of the muscles supra-maximal nerve stimuli. The duration of the potentiation is brief in contrast to that produced by physostigmine, neostigmine, and DFP. Varying degrees of depression of the muscular response to supra-maximal nerve stimuli are produced by large doses of Nu 2017. The recovery from depression is rapid and the return to normal from a completely refractory state occurs within 20 minutes. After inactivation of cholinesterase by DFP, the close intra-arterial injection of a large dose of Nu 2017 produces a profound depression from which recovery is prolonged. The depression of the response to indirect stimuli produced by d-tubocurarine is antagonized by Nu 2017.

Preparation and pharmacological properties of the acid succinate of myanesin. RICHARD F. RILEY and F. M. BERGER (introduced by HAROLD C. HODGE). *Depts. of Pharmacology and Pediatrics, Univ. of Rochester, Rochester, N. Y.* Observations on the metabolic fate of myanesin led to the assumption that myanesin acid succinate would represent a soluble, relatively non-toxic derivative of myanesin with a less intense and longer duration of action. In the present investigation, the succinate was prepared and tested and found to behave in accordance with this expectation. The succinate, prepared by reacting myanesin with succinic anhydride in pyridine, was obtained as straw-colored, extremely viscous liquid which gives a highly water soluble sodium salt. In the mouse, the paralytic and lethal doses of sodium myanesin succinate are found to be larger than those of myanesin. The antagonism and duration of action of these two drugs to metrazole convulsions was studied by the injection of these compounds before and after the administration of an LD99 of metrazole. On a molar basis, the succinate is less effective in preventing convulsions when administered jointly with metrazole. The succinate is, however, equally effective when administered prior to the convulsant. The succinate in small doses abolishes tremors and hyperexcitability due to strychnine without abolishing the knee jerk in the cat.

Failure of alpha tocopherol to influence chest pain in patients with heart disease. SEYMOUR H. RINZLER (by invitation), HYMAN BAKST (by invitation), ZACHARY H. BENJAMIN (by invitation), AUDRIE L. BOBB (by invitation), and JANET TRAVELL. *Cardiovascular Research Unit of Beth Israel Hospital and the Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* A stringent blind-test method, in which neither patients nor examiners knew whether alpha tocopherol or a matching placebo was being administered, was used to investigate effects of alpha tocopherol on long standing chest pain in 38 ambulatory clinic patients with heart disease (chiefly arteriosclerotic and hypertensive). For allotment to control and treated groups, patients were paired with respect to sex, age, cardiovascular status, duration and type of chest pain, and other pertinent factors. One of each pair was assigned to either control (placebo) or treated (vitamin) groups. Types

of chest pain encountered were effort angina, constant chest pain, and intermittent chest pain unlike effort angina. We regard the first group as primarily of cardiac origin, while the other two are considered chiefly somatic. The dosage plan was: 200 mg. of synthetic alpha tocopherol (Ephynal Acetate 'Roche') by mouth daily for 2 weeks and thereafter 300 mg. daily. The average duration of vitamin administration was 16 weeks (10–20 weeks), and of the placebo 16.6 weeks (10–20 weeks). Response to medication was similar for both groups. No improvement was noted in 12 treated subjects (63%) and 14 controls (73%). Subjective improvement was reported by 7 treated patients (37%) and 5 controls (27%). Serial electrocardiograms, exercise tolerance tests, repeated blood pressure readings and measurements of skeletal muscle function did not reveal significant differences between treated and control groups. Our results fail to confirm the reported benefits of alpha tocopherol in cardiac pain.

Pharmacologic and sensitizing properties of SY-28 when administered by ion transfer. ARTHUR A. RODRIQUEZ (by invitation), JULES H. LAST, and ADOLPH ROSTENBERG JR. (by invitation). *Depts. of Physical Medicine, Pharmacology, Dermatology and Allergy Unit, Univ. of Illinois College of Medicine, Chicago, Ill.* The pharmacologic activity of SY-28 (N-ethyl-N-(1-naphthylmethyl)-β-bromoethylamine hydrobromide) was studied following administration by ion transfer to the normal skin of human subjects. Three-tenths of a milliampere of anodal current was applied to 10 cm.² areas of the skin. A total amount of 0.3 cc. of a 10 mg. % solution of SY-28 in propylene glycol was used. It was observed that SY-28 when administered in the above manner was a strong eczematous sensitizer. Twelve out of 46 patients (26%) became sensitized to the drug. Adrenergic blocking activity lasting at least 24 hours was demonstrated in 53 patients. Antihistaminic activity was also noted following administration of SY-28. There was clearly no interference with the pharmacologic action of SY-28 in those individuals who became sensitized.

Interaction between hematin and serum albumin. MORRIS ROSENFELD and DOUGLAS M. SURGENOR (by invitation). *Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins School of Medicine and Dept. of Physical Chemistry, Harvard Medical School, Boston, Mass.* The reaction between hematin and human serum albumin to form methemalbumin has been studied by spectrophotometric procedures. Titration of hematin with albumin reveals a one to one molar ratio at the endpoint. Other interactions involving simple stoichiometric relationships are indicated by titration curves. Little interaction is observed spectrophotometrically with other plasma protein fractions including fibrinogen and the alpha, beta and gamma globulins. A direct colorimetric procedure for the estimation of serum albumin has been based on the hematin reaction. The colorimetric measurements compare favorably with values obtained by the low temperature methanol fractionation method of Pillemer and Hutchinson.

Thiomerin: toxicity and diuretic effects. A. RUSKIN, J. E. JOHNSON, W. N. RODDY (introduced by CHAUNCEY D. LEAKE). *Heart Station, Univ. of Texas Medical Branch, Galveston.* The disodium salt of N (γ-carboxymethylmercaptomercuri-B-methoxy) propyl camphoramic acid (thiomerin) in isolated rabbit hearts causes A-V and I-V block and asystole in doses 20 times those of meralluride (mercuhydrin), and about 50 times those of mersalyl-theophylline and mercuzanthin. In 50 clinical cases the diuretic effects of 2 cc. doses of thiomerin subcutaneously are approximately equivalent to those of 2 cc. doses of mercuhydrin intravenously, both containing the same amount of mercury (80 mg.) in organic combination. Cases in which mercuhydrin produced untoward effects (nausea, vomiting, muscle cramps, toxic psychoses), tolerated thiomerin well. Rare local soreness and nodulation from thiomerin is now being investigated by similar comparative methods.

Adrenergic blocking agents and the vasopressor response to epinephrine. DAVID RUSSELL (by invitation) and GRAHAM CHEN. *Research Labys., Parke, Davis & Company, Detroit, Mich.* Quantitative results on 'epinephrine reversal' of blood pressure in the dog have been obtained with two adrenergic blocking agents: N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine·HBr and yohimbine. The data were found to conform to either Langmuir's adsorption isotherm or Gaddum's equation of competitive inhibition based on the law of mass action. A linear relationship is obtained when the degree of 'epinephrine reversal' is expressed in terms of the percentage of inhibition of epinephrine in probit units and the doses of the adrenergic blocking agent in logarithms.

Action of cholinesterase inhibitors on the isolated rabbit ileum. P. R. SALERNO (by invitation) and J. M. COON. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* It has been reported by us (*Fed. Proc.* 7: 212, 1948) that physostigmine, neostigmine, TEPP, and DFP, in molar concentrations of approximately 10^{-8}, change the pendular movements of the isolated rabbit ileum to a peristaltic rhythm. The appearance of moving constriction rings in the muscle strip characterizes this change. The peristaltic rhythm can be reversed to a pendular rhythm by atropine and thiamine, or by the ganglionic blocking agents procaine, nicotine, tetraethyl ammonium and magnesium. Effective concentrations of the latter

blocking agents have no effect upon the sensitivity of the gut to acetyl choline added to the bath. The change in rhythm cannot be induced in the untreated muscle by acetylcholine itself. Gut preparations kept at 1°C. for 2 days exhibit a typical pendular activity when placed in oxygenated Ringer-Locke at 37.5°C. and respond to the esterase inhibitors by an increase in tone but not by the usual change in rhythm. After four days the gut is totally insensitive to the cholinesterase inhibitors and nicotine but responds to acetylcholine, pilocarpine, barium, and epinephrine. The cholinesterase activity of rabbit ileum was found to be stable at 1°C. for at least 25 days. It is believed that the conversion from pendular to peristaltic rhythm induced by the cholinesterase inhibitors in the isolated gut depends upon the endogenous synthesis of acetylcholine and the integrity of the intrinsic ganglion cells. The loss of sensitivity in the old intestinal strips might be caused by a degeneration of the ganglion cells and a cessation of acetylcholine synthesis.

Inotropic synergism between digitalis bodies and serum calcium. WILLIAM T. SALTER, JOHN GEMMEL (by invitation) and LOUIS SCIARINI (by invitation). *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.* The hypocalcemic frog heart can be employed with a few ml. of human serum or Locke's solution to test the inotropic potency of a cardiac glycoside. By adjustment of the concentration of Ca^{++} ions, graded stages of hypodynamic contraction can be produced seriatim. Such preparations at *pH* 7.3 follow the law: $R/(100 - R) = [Ca^{++}] - (A - k[G])$, where R is the percentile contractile response of the heart, $[Ca^{++}]$ the concentration of calcium ions, A the individual 'heart constant', k the potency of the cardiac glycoside and [G] its concentration. Each preparation is calibrated in standard mammalian Ringer's solution to determine the 'heart constant' A. Then the $[Ca^{++}]$ is adjusted and the response R measured when the test heart has been equilibrated with the dissolved preparation or serum under test. Finally the value k[G] is calculated. When R = 50%, $k[G] = A - [Ca^{++}]$. In terms of ouabain, it is difficult to avoid toxic effects at concentrations higher than 5 micrograms %, even when the $[Ca^{++}]$ is low.

Isopropyl alcohol as an anticonvulsant. RALPH W. SCHAFFARZICK (by invitation) and P. J. HANZLIK. *Dept. of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco 15, Calif.* The minimum effective blood acetone for anticonvulsant action (cortical threshold by electrical stimulation) in rats receiving acetone gastrically was found to be about 50 mg. % which could be nearly doubled and the threshold raised 100% with excessive doses, without ataxia. The minimum effective anticonvulsant blood concentration of alcohol, after isopropyl alcohol gastrically in rats, was markedly irregular. After 500 mg. and 1250 mg/kg. doses of isopropyl alcohol gastrically, the increase in cortical threshold paralleled more closely the blood alcohol than the blood acetone. When the higher dose of the alcohol was given in 3 divided doses in 12 hours, the acetonemia was cumulative with peak increases in cortical threshold in 1 hour, recovery at end of 12 hours, and suggestions of tachyphylaxis to the cortical stimulus (rats and rabbits). Generally the acetonemia outlasted the anticonvulsant action (6-hour observations). Only about 3% (average) of the higher dose of the alcohol was excreted in 24 hours in the urine of rabbits, the ratio of excreted alcohol to acetone being about 2.3 to 1. The share of alcoholemia and acetonemia in the anticonvulsant action of isopropyl alcohol remains undetermined. Surely convulsant but generally not fatal doses of metrazol (50–70 mg/kg.) and picrotoxin (2–3 mg/kg.) subcutaneously were partially antagonized by the higher dose of isopropyl alcohol gastrically in the great majority of rats.

Effect of some vasodilators and sedatives on gastric secretion. ROBERT B. SCHLESINGER (by invitation), F. STEIGMANN and LEO L. HARDT (by invitation). *Hektoen Inst. for Medical Research of Cook County Hospital and the Dept. of Therapeutics of Cook County Hospital and the Depts. of Internal Medicine, Univ. of Illinois, College of Medicine and Stritch School of Medicine, Loyola Univ., Chicago, Ill.* Because some patients have epigastric discomfort after the ingestion of some vasodilator and sedative drugs, the effect of such substances on gastric secretion was studied on 32 patients (21 with duodenal ulcer and 11 controls). The gastric contents were aspirated every 15 minutes and returned to the stomach except 10 cc. which were used for titration for gastric acidity. Following the fourth aspiration one of the drugs to be tested was administered. The following drugs were tested; *1*) aminophylline, gr. VI enteric coated; *2*) aminophylline, gr. III plain; *3*) sodium nitrite, gr. I; *4*) glucophylline, 4.68 gr.; *5*) nicotinic acid, 100 mg.; *6*) butisol tablets, gr. ISS (sodium 5-ethyl-5-sec-butyl barbiturate); *7*) elixir of butisol fl. oz. 1 (gr. III) given orally; *8*) aminophylline, gr. 7*2* and *9*) glucophylline .36 gm. administered intravenously. Aminophylline and glucophylline intravenously and tablets and elixir of butisol increased the free acid, the total HCl and the volume. Nicotinic acid and aminophylline tablets (gr. VI) increased the volume and total HCl. Glucophylline, aminophylline (gr. III), and sodium nitrite decreased the free acid, the volume and the total HCl in all but 3 patients. Since aminophylline and glucophylline orally did not increase acidity, increased free acid following intravenous aminophylline (average 21 units) and

glucophylline (average 10 units) may result from central stimulation. The epigastric distress in some individuals is probably due to other causes than increased gastric acidity, although results of single gastric analyses must be cautiously interpreted.

Fluorimetric vs. biologic assays (human and animal) of fractions of digitalis purpurea. LOUIS SCIARINI (by invitation) and WILLIAM T. SALTER. *Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.* Tincture of digitalis has been fractionated into 5 arbitrary extracts, based largely on differential solubility in chloroform and rather concentrated solutions of sulfuric acid. To each of these a fluorimetric procedure has been applied and the value compared wirh Harry Gold's human oral assay and/or with well-substantiated assays by the intravenous cat or pigeon methods. The relative significance of each fluorimetric value, measured against crystalline digitoxin as a standard, is given by the conversion factor F, when F × the fluorimetric value = biological activity. Even so, a correction must be made for gitoxin. The oral human activity resides almost exclusively in fraction V. The data suggest that the oral potency of digitalis purpurea is due solely to its digitoxin content. For our fraction V, a series of Harry Gold's preparations (in terms of digitoxin per gram of powdered leaf) gave: *A*) by Gold's human assay; *a*) 1.14; *b*) 1.15; *c*) 1.08; *d*) 1.43 and *e*) 1.02, respectively; *B*) by fluorimetry *a*) 1.14; *b*) 88.6; *c*) 1.09; *d*) 1.43 and *e*) 1.07, respectively. Much of the biological activity after intravenous injection of lethal doses into cats or pigeons occurs in fractions II and III. For example, in one preparation the percentile lethal activity (pigeons) in various fractions was: *I*) 36%; *II*) 21%; *III*) 14%; *IV*) 7% and *V*) 21%. For such values, a series of conversion factors (based on intravenous, animal, lethal effects) can be determined.

Distribution and excretion of beryllium sulphate using Be^7 as a tracer. JAMES K. SCOTT and WILLIAM NEUMAN (introduced by H. C. HODGE). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* Beryllium sulphate was administered intravenously to rats and rabbits; the rate of blood clearance, the urinary and fecal excretion and the distribution to organs was determined by counting techniques. In some cases nonradioactive beryllium sulphate was added to the isotopic beryllium sulphate. More than 95% of the beryllium was cleared from rabbits' blood during the first 5 hours after administration; the rate of clearance was more rapid when isotope alone was administered than when carrier was added. The urinary excretion of beryllium was greatest during the first 6 hours after administration, thereafter small amounts, less than 1%, were excreted daily. In both rabbits and rats a higher % of the isotope was excreted when isotope alone was given than when carrier was added: 28% and 11%, and 35% and 25% respectively. The total fecal excretion over a 7-day period was approximately 10% of the dose in the rats and 2% in the rabbits. When carrier was administered with the isotope approximately 35% was found in the skeleton of the rat and 51% in the skeleton of the rabbit; when isotope alone was administered the skeletal recovery was 48% and 54%. The amount of isotope recovered from the liver and spleen of the rat was insignificant when isotope alone was administered and amounted to 12% and 2.5% respectively when carrier was added. Significant amounts of beryllium were present in the bone marrow when carrier was administered with the isotope. The kidneys contained less than 1% of the dose.

Tachyphylaxis of sympathomimetic drugs. LLOYD D. SEAGER and WILLIAM WEISS (by invitation). *Depts. of Pharmacology and Anesthesiology, Woman's Medical College of Pennsylvania, Philadelphia, Penna.* A comparison was made of a number of sympathomimetic drugs as to their property of showing tachyphylaxis. Blood pressure response in vagotomized dogs and cats was the function studied. Of the drugs studied, the phenomenon was found to occur most readily with desoxyephedrine. In some experiments, complete tachyphylaxis with reversal was found following an initial dose of 1 mg/kg. Oenethyl, propadrine, benzedrine, ephedrine and paredrine were next in order of susceptibility. As tachyphylaxis developed to any of these agents, the animal was found to show diminished or no response to the others of the group. A reversal of blood pressure after repeated dosage occurred most readily with desoxyephedrine and oenethyl but could be produced with large doses of the other compounds. Reversal was usually associated with some slowing of the pulse. Some diminution of the response to neosynephrine, and epinephrine was found after massive doses of ephedrine, desoxyephedrine, benzedrine, oenethyl, and propadrine. Nicotine was usually found to give some increase in blood pressure after complete tachyphylaxis had developed to one of the sympathomimetic agents.

Epinephrine and dibenamine in the alarm reaction. JOSEPH SEIFTER, WILLIAM E. EHRICH, ALBERT J. BEGANY (by invitation), and GEORGE M. HUDYMA (by invitation). *Wyeth Inst. of Applied Biochemistry, and the Graduate School of Medicine of the Univ. of Pennsylvania, Philadelphia, Penna.* Colchicine is considered the most potent stimulus available for provoking the alarm reaction, characterized by involution of lymphoid tissue and shock. Since discharge of epinephrine is thought to be the first consequence of exposure to an alarming stimulus, it was of interest to determine whether the administration of epinephrine en-

hances the toxicity of colchicine and whether the administration of an adrenolytic drug decreases the toxicity of colchicine. Results; *1*) epinephrine is a less intense alarming stimulus than colchicine; *2*) dibenamine (dibenzyl-β-chloroethylamine·HCl) produced a mild alarm reaction, which subsided in 24 hours; *3*) non-lethal doses of epinephrine combined with non-lethal doses of colchicine resulted in fatality, with extreme alarm reaction; *4*) dibenamine protected against the lethal effects of either colchicine or epinephrine, but not against the combination of colchicine and epinephrine; *5*) the histological changes of the alarm reaction in the thymus and the adrenals provoked by epinephrine were reversed by dibenamine, but those provoked by colchicine were not.

Effects of certain central nervous system depressants and stimulants on intermediary metabolism of brain. F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* The results presented here are preliminary data obtained from experiments designed to determine differences in biochemical behavior of 'stimulants' (convulsants) and 'depressants' (anesthetics) as judged by their *in vitro* actions on isolated central nervous system tissue. The effects of a series of central depressants and stimulants on no-substrate oxygen consumption of rat cerebral mince were determined. In no case was there obtained any significant effect other than inhibition. Excellent correlation between relative *in vitro* and *in vivo* potency was obtained in the depressant series of drugs but was not apparent in the stimulant series. A representative member of each group (chloral hydrate and metrazol) was employed for further studies in concentrations which had produced equal inhibitions of the Q_{O_2} of rat cerebral mince. The degree of inhibition of the no-substrate Q_{O_2} of brain mince with both compounds paralleled *a*) the degree of specialization of the tissue and *b*) the phylogenetic development of the animal species studied. With glucose, lactate, pyruvate, α-ketoglutarate and fumarate, Δ-substrate oxygen consumptions of rat cerebral cortex mince were equally well inhibited by both chloral hydrate and metrazol. When succinate was added as a substrate the 2 drugs showed a quantitative difference in their effects. Both compounds had the same qualitative effect on anaerobic glycolysis of rat cerebral mince but differed quantitatively. Whereas metrazol increased anaerobic glycolysis 25%, chloral hydrate effected a 100% increase in the $Q_G^{N_2}$.

Production of ventricular extrasystoles in the dog by a polypropylene glycol. F. E. SHIDEMAN and G. K. MOE. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* A polypropylene glycol with an average molecular weight of 750 produced ventricular extrasystoles when it was administered intravenously in the anesthetized dog. The total amount required to produce the extrasystoles varied (10 mg/kg. to 90 mg/kg) from animal to animal but the duration appeared to depend on the total amount of polyglycol administered rather than on the size of a single dose. In no experiment was it possible to produce ventricular fibrillation either by further administration of polyglycol or by epinephrine, once extrasystoles had been established. Bilateral vagotomy in no way influenced the extrasystoles. Epinephrine or tetraethylammonium chloride in some cases caused transient disappearance of the cardiac irregularity, but not in others. Administered in divided doses in the heart-lung preparation, the polyglycol produced increasing degrees of decompensation and finally complete failure. In this preparation, and in artificially respired animals with the heart exposed, extrasystoles were never produced by the polyglycol alone or combined with peripheral vagal or cardiac sympathetic stimulation. However, when in the open chest preparation, the respiratory volume was reduced to a point where the animal displayed respiratory efforts, extrasystoles were readily induced and could be abolished by increasing the respiratory volume. Such findings would suggest that the sensitivity of the heart to this polypropylene glycol is dependent in some way on a relative anoxia.

Distribution effect and fate of oil aerosol particles after retention in the lungs of mice. MILTON SHOSHKES, WILLIAM G. BANFIELD, JR., and S. JOSEPH ROSENBAUM (introduced by AMEDEO S. MARRAZZI). *Toxicology, Pathology, and Aerosol Sections, Medical Division, Army Chemical Center, Md.* Mice were exposed to mists of corn, peanut, cod liver, mineral, and motor oils, with an average mass median diameter of 2.5μ in dynamic chamber-type experiments. The general dispersion of the retained oil droplets in the lung was found to be equal from apex to base and from hilus to pleura, and the highest concentration was noted in and around the terminal bronchioles and alveolar ducts. In a statistical analysis of the distribution and size of corn oil particles retained in the lung, it was noted that 80% of all the particles retained in all areas of the lung were 2.5μ or less in diameter. The initial concentrations of retained oil droplets in the lung after exposure to all the oil mists were similar. However, during a 96-hour period of observation, the concentration of the edible oils decreased progressively, while the concentration of the paraffin oil droplets remained essentially unchanged. Except for the appearance of scattered macrophages, no acute inflammatory changes were seen after a 2-hour exposure to these oil mists within a 96-hour observation period. After heavy exposures, mineral and motor oil droplet retention resulted in localized foreign body reactions of moderate severity as well as infrequently occurring

patches of lipid pneumonia. Cod liver oil droplets caused a moderate foreign body reaction and only negligible reactions to corn and peanut oil were seen.

Effect of renal dysfunction on the urinary excretion of fluoride in the rabbit. F. A. SMITH and D. E. GARDNER (introduced by H. C. HODGE). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* The effect of uranium-produced nephritis on the urinary excretion of ingested fluoride has been studied in rabbits receiving: *1)* a subcutaneous injection of 0.3 mg. U/kg as uranyl nitrate; *2)* the same dosage of uranium in addition to 15 ppm fluoride in the drinking water, and; *3)* 15 ppm fluoride in the water supply. Control urinary fluoride excretion was determined. Inspection of the analytical data shows that within 3 to 4 days after injection of the nitrate, the urinary excretion of fluoride decreased to a level only ¼ as great as obtained prior to the uranium injection. Twelve days after the injection, the urinary fluoride level was again normal in those rabbits which were not receiving fluoride in the drinking water; only 4 days were required for the return to normal levels of urinary excretion in those animals whose water supply contained added fluoride. Fluoride content of the tooth and bone indicated that the presence of uranium in the kidney may inhibit the deposition of ingested fluoride in the bone and thus offer a degree of protection against fluorosis. The fluoride contents of the tooth root, femoral epiphysis and jaw alveolar bone for rabbits receiving only added fluoride were significantly higher than in comparable tissues from rabbits treated with both uranium and fluoride, despite the greater quantity of fluoride consumed by these animals. Other supporting evidence for the protective effect of added fluoride on uranium poisoning was: *1)* a 2.5-fold lower blood N and; *2)* lowered mortality.

Rate of removal of alcohol in acutely intoxicated chronic alcoholics. H. W. SMITH and R. G. BELL (introduced by J. K. W. FERGUSON). *Dept. of Pharmacology, Univ. of Toronto and Shadowbrook Health Foundation, Toronto, Canada.* The rate of removal of alcohol from the blood of 21 chronic alcoholics prior to treatment with intravenous insulin (40 U zinc insulin), glucose (50 ml. of 50%) and 'Betalin' (Lilly, 2 ml.) was compared to the rate during treatment. This rate was increased during treatment by 9.9% ($S^- = 3.3\%$) when the rate was calculated on the basis of % of the amount of alcohol present. Seven untreated patients showed no change in the hourly rate of removal of alcohol from the blood during comparable periods of study.

Effects of tetraethylammonium chloride on pain thresholds in man. RALPH R. SONNENSCHEIN (by invitation), ELIZABETH H. JENNY (by invitation) and CARL C. PFEIFFER. *Depts. of Pharmacology and Clinical Science, Univ. of Illinois College of Medicine, Chicago 12, Ill.* The intravenous injection of 500 mg. of tetraethylammonium chloride (9 trials on 4 subjects) raised significantly the pain threshold of the finger pad, lowered that of the nail bed, and left practically unaffected that of the tooth. Thresholds of the nail bed and pad of the 3rd finger were determined by a radiant heat device. Tooth thresholds were determined by electrical stimulation of the pulp through a metal filling; both the perceptive threshold (TI) and affective threshold (TII) were measured. Over a 30-minute period following injection, pad threshold showed an average maximum rise of 14% ($P < 0.001$); at this point, 10 minutes after injection, the nail threshold was decreased by 6% ($P < 0.02$). No consistent, significant change was seen in tooth thresholds. Preliminary experiments (Pfeiffer et al.: *Ann. N. Y. Acad. Sci.* 51: 21, 1948) reveal that procaine block of the stellate ganglion, with Horner's syndrome, causes a rise in nail and fall in pad threshold. It therefore is unlikely that the above action of TEA is due to ganglionic blockade. Block of the median nerve with 0.5% procaine causes a rise in pad and fall in nail threshold. The effect of TEA is another example of this reciprocal relationship. The evidence indicates that pain of nail bed and pad is mediated by 2 separate mechanisms. The primary action of TEA in raising pad threshold may be through a selective peripheral or central effect on the underlying mechanism for pain from this area.

Clinical criteria in beryllium poisoning. C. J. SPIEGL, F. A. SMITH, L. J. LAFRANCE, J. W. KEATING (introduced by H. C. HODGE). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* At present no reliable biochemical methods exist for the diagnosis of early or mild forms of beryllium poisoning. Accordingly a broad survey of clinical criteria was made in animals undergoing exposure. This included urinary protein and amylase, blood nonprotein nitrogen, urea nitrogen, serum protein, serum albumin-globulin ratio, fibrinogen, serum calcium, phosphorus, acid and alkaline phosphatase, arterial oxygen tension, 10 lipid fractions of the blood with special attention to phospholipid and free cholesterol concentrations in the red cells. Dogs and rabbits were studied that had been exposed to beryllium sulfate at concentrations ranging from 1 to 100 mg/m³ and beryllium oxide at from 10–85 mg/m³ for periods up to 100 days. Of the many criteria applied, arterial oxygen tension and red cell concentrations of free cholesterol and phospholipid give indication of potential value as clinical procedures in following the course of beryllium injury. Many of the other criteria failed at lower levels of injury.

Cocaine-barbiturate antagonism. JOHN E.

STEINHAUS (introduced by A. L. TATUM). *Dept. of Pharmacology, Univ. of Wisconsin Medical School, Madison, Wis.* A quantitative study of cocaine-barbiturate antagonism was undertaken to evaluate the effectiveness of prophylactic doses of barbiturate for toxic reactions of local anesthetics. A series of young, male, New Zealand, white rabbits (1.5 to 2.5 kg.) were injected subcutaneously with doses of cocaine ranging from 100 to 300 mg/kg. They were then treated intravenously with various doses of pentobarbital at different time intervals with respect to the cocaine injection. It was found in these studies that higher doses of cocaine required correspondingly higher doses of pentobarbital. With symptomatic treatment the average dose of pentobarbital required for rabbits receiving 300 mg/kg. of cocaine was almost double that required for animals in the 100 mg/kg. series. The time of pentobarbital administration for maximal effectiveness was at the time of cocaine injection. Treatment 45 minutes before or 15 minutes after this time was ineffective. When 100 mg/kg. (the generally accepted LD-50) of cocaine was given subcutaneously, 15 mg/kg. of pentobarbital resulted in 100% survival, whereas 5 mg/kg. gave no protection. These data suggest that the protection afforded by barbiturate depends upon the degree of depression and that approximately ⅓ of the anesthetic dose is required for adequate protection. This would make it appear questionable that the commonly used 'prophylactic' dose (0.1 to 0.2 gm. orally) gives adequate pharmacodynamic protection.

Comparison of the acute toxicity of hydroquinone and some related di and trihydroxy benzenes. JAMES H. STERNER (by invitation), STANLEY AMES (by invitation) and DAVID W. FASSETT. *Eastman Kodak Company, Rochester, N. Y.* Recent developments in use of polyhydroxy benzenes as antioxidants in fats for human consumption have stimulated an interest in their relative toxicologic properties. The acute oral and parenteral toxicity of hydroquinone (HQ), nordihydroguaiaretic acid (NDGA), n-propyl gallate (NPG) and butylated hydroxy anisole (BHA) reveals certain marked differences with respect to onset and character of symptoms, time of death, and a wide spread between the ratio of parenteral and oral LD_{50} values. In the white rat, the approximate LD_{50} in mg/kg. intraperitoneally were: NDGA—70, HQ—168, BHA—250, NPG—250; orally, the values were HQ—370, NDGA—2400, NPG—2500, BHA—2500. The rapid onset of symptoms and the occurrence of death within 30 minutes with hydroquinone contrasted sharply with the delayed onset of symptoms and marked variability in time of death with the other antioxidants which varied from a few minutes to 7–10 days. The apparent recovery of the hydroquinone survivors was very rapid, a matter of an hour or two, compared with the prolonged inanition and debility of the survivors of the animals given NDGA, BHA, and NPG. These results suggest a fundamental difference in excretion and metabolism of hydroquinone (a normal excretory product in urine) and the compounds of more complex structure.

Pharmacologic action of chlordane. E. F. STOHLMAN, W. T. S. THORP and M. I. SMITH. *Experimental Biology and Medicine Inst., National Insts. of Health, Bethesda, Md.* The acute toxicity, LD_{50} of chlordane in rats was found to be approximately 250 mg/kg when given orally in olive oil or Tween 20. The LD_{50} of DDT under similar conditions is 150 mg/kg. On intraperitoneal injection chlordane in Tween 20 was slightly more toxic than when given in olive oil. In rabbits the LD_{50} of both compounds is approximately 300 mg/kg. Death from chlordane poisoning may be delayed for many days. Chronic toxicity of chlordane was considerably greater in both rats and rabbits than that of DDT under similar conditions, probably due to greater cumulative effects of chlordane. Repeated administrations of chlordane orally produced in rabbits focal necrosis of the liver, degenerative changes in the proximal convoluted tubules of the kidneys, and pulmonary exudates in the alveoli and bronchioles of the lungs, whereas with large single doses the primary effect appeared to be on the lungs, producing focal hemorrhages. Organically bound chlorine was excreted in the urine of rabbits to the extent of about 18% of the dose given, most of it during the first 4 days following administration. Significant amounts of organic chlorine were excreted in the urine of rabbits following .25 mg/kg or more chlordane.

Acute inhalation toxicity of beryllium sulfate in animals. H. E. STOKINGER, G. F. SPRAGUE, R. H. HALL, J. K. SCOTT, F. A. SMITH, B. H. BROWN, and A. PETTENGILL (introduced by H. C. HODGE). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* The acute toxicity from inhalation of beryllium sulfate mist in animals was determined in large-scale experiments wherein the toxic limits of exposure and the characterization of the acute response were established. A series of 4 concentrations (100, 50, 10 and 1 mg $BeSO_4 \cdot 6H_2O$/cu.m. of air) were tested in daily 6-hour exposures for periods of from 14–105 days. Particle size of the mist was from 0.5 to 1.3μ. Marked species variation in response was noted. At levels of 100 and 50 mg/m^3, 80–100% mortality occurred during week 1–6 in the cat, dog, rat, goat and monkey, 60% (week 1–2) in the guinea pig, 10–50% (week 2–5) in the rabbit, mouse and hamster; all swine and fowl survived. Over-all mortality, chiefly confined to the rat, at 10 mg/m^3 was 14%; at 1 mg (0.04 mg Be/m^3) no mortality was observed in any species and weight losses were recovered at the

end of 90 days' exposure although certain evidences of poisoning were still present. The monkey, dog, cat and rabbit showed acute pulmonary inflammatory response, resembling that in man; the rat gave an atypical response. Effects that were severe and rapid in onset at the 100 and 50 mg.-levels were minimal and slower in developing at the 10 mg.-level changing to moderate with continued exposure and, at the 1 mg-level, were nonexistent in certain species at the end of 105 days' exposure. Highest levels produced severe proteinuria and changes in blood protein values, not apparent at lower levels. At the lowest level, reduced blood oxygen tension and alteration in blood cell lipid ratios occurred. A hypochromic macrocytic anemia appeared in the dogs. Beryllium concentrations in 20 tissues were determined. A control study with $NaHSO_4$ mist showed that the lesions produced in $BeSO_4$ exposures were due to beryllium and not to the sulfate ion.

Protective effect of certain drugs on epinephrine-induced pulmonary edema. CLEMENT A. STONE (by invitation) and EARL R. LOEW. *Dept. of Physiology, Boston Univ. School of Medicine, Boston, Mass.* Studies were made with certain drugs to determine their ability to prevent the pulmonary edema in rabbits following an intravenous injection of 0.375 mg/kg. of epinephrine as the base. Protective drugs were injected intravenously 10 minutes prior to the epinephrine. The indication of efficacy was the presence of a statistically significant reduction in lung weight and/or mortality from that of a series of concurrent control experiments. Previous reports (Luisada: *Arch. f. exper. Path. u. Pharmakol*, 132: 313, 1928) indicate that hypnotics in general are effective in reducing mortality due to epinephrine. Pentobarbital sodium (25.0 mg/kg.) in our experiments was ineffective in preventing the hemorrhage and edema of the lungs, which is the usual cause of death after large doses of epinephrine. Atropine sulfate (5.0 mg/kg.) significantly diminished the increase of lung weight, but not mortality, as compared to controls, but was not as effective as N-(2-chloroethyl)-N-ethylbenzhydrylamine·HCl (SY-2, 2.5 mg/kg.) or N-(2-bromoethyl)-N-ethyl-1-naphthalenemethylamine·HBr (SY-28, 0.25 mg/kg.). These drugs are potent adrenergic blocking agents; SY-28 having, in addition, a marked ability to antagonize histamine. The complete protection afforded by SY-2 and SY-28 is probably related to their ability to annul the acute hypertensive action of epinephrine. The failure of Neoantergan (2.5 mg/kg.), a potent and specific antihistaminic, to protect indicates that histamine does not play a prominent role in the edema formation. The protective effect of relatively large doses of the anti-histaminic, Phenergan or 3277 R.P. (*Acta allergologica*, 1: 97, 1948) may be related to other properties, among which is an appreciable atropine-like action.

Hypotensive activity of fractions from green Hellebore. J. W. STUTZMAN, GEORGE L. MAISON and G. W. KUSSEROW (by invitation). *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass. and Product Development Dept., Rexall Drug Company, Los Angeles, Calif.* Fifty fractions of green hellebore were tested for hypotensive activity in normotensive dogs which were unanesthetized or lightly anesthetized with pentobarbital sodium. On intravenous administration the fractions were found to fall into three groups on the basis of hypotensive action. One group did not alter mean arterial pressure; a second caused a gradual minimal fall; and a third an abrupt fall with slow recovery over several hours. The last group included both water soluble and organic solvent fractions. In this group hypotension was accompanied by bradycardia of short duration. Minimal effective intravenous dosage was 0.002 mg/kg from a fraction which represented 0.35% of the crude drug. Potent fractions were effective orally at a dosage of 0.2 mg/kg. Toxic effects of high doses included retching, emesis, apnea, hypertension and convulsions.

Comparative anticonvulsant potencies of some phenylhydantoins and their corresponding phenylacetylureas. EWART A. SWINYARD and JAMES E. P. TOMAN. *Depts. of Pharmacy, Pharmacology and Physiology, Univ. of Utah College of Medicine and College of Pharmacy, Salt Lake City, Utah.* Anticonvulsant potencies (ED_{50}) of 5-phenyl-5-R-hydantoins (h) and their corresponding phenyl-R-acetylureas (a) were determined by their ability to modify maximal electroshock seizure pattern and to prevent Metrazol convulsions in rats. Drugs were given orally in 10% neutral acacia suspension and tested at the previously determined time of peak drug effect. Minimal neurological toxicity (TD_{50}) was determined, and protective indices (P. I. = TD_{50}/ED_{50}) were calculated. Maximum potency occurred with alkyl substitutions. Unsubstituted phenylacetylurea (Phenurone) was superior to its hydantoin homolog, whereas diphenylacetylurea was inactive in contrast to diphenylhydantoin (Dilantin). Studies on the metabolic fate of these compounds might help to identify a common denominator as the effective product.

Purification of diamine oxidase (histaminase). HERBERT TABOR. *Experimental Biology and Medicine Inst., National Insts. of Health, Bethesda, Md.* A highly purified, stable diamine oxidase has been obtained from an extract of hog kidney acetone powder with the aid of a new colorimetric procedure for the estimation of histamine (Rosenthal and Tabor, *J. Pharm. Exp. Therap.*, 92: 425, 1948). The acetone powder was extracted with

0.2M phosphate buffer *pH* 7.2, and fractionated with Na_2SO_4 (14.5–21%). This fraction was dialyzed, heated to 60°C. for 20 minutes, adsorbed on alumina gel Cγ, and eluted with M K_2HPO_4. The dialyzed eluate was fractionated with ethanol at 0°C., and dried *in vacuo*. This dry preparation is 500 times more active per mg. protein than the original extract, and represents a yield of approximately 25%. 0.006 mg. of this purified powder destroys 0.03 mM of histamine in 5 minutes in an incubation mixture containing 0.1mM of histamine and 1.3 mg. of crystalline bovine albumin (as a protective protein) in 3 cc. of 0.1 M phosphate buffer *pH* 7.2 (37°C.). Further purification can be obtained by adsorption on calcium phosphate gel and elution with phosphate buffer. The action of diamine oxidase on histamine probably results in destruction of the imidazol ring, since the products give no color with diazotized p-nitro aniline. In the destruction of putrescine and cadaverine, neither succinic acid nor γ-amino valeric acid, respectively, could be detected. The mechanism of breakdown of these diamine compounds is being further investigated.

Comparative study of the local toxic action of thiomerin, mercuzanthin and mercuhydrin. HARRY TAUBE (by invitation), Robert A. LEHMAN and E. E. KING (by invitation). *Dept. of Therapeutics, New York Univ. College of Medicine, New York City.* The mercurial diuretic thiomerin, (di-sodium salt of N-[gamma-carboxymethyl-mercaptomercuri, beta-methoxy] propylcamphoramic acid), has been compared with mercuzanthin and mercuhydrin with respect to the gross pathology produced following subcutaneous injection in the abdomen of the mouse and the histopathology occurring after intramuscular injection in the Tibialis anterior muscle of the rat. It was found that thiomerin was tolerated by the mice without gross pathologic changes while the other 2 drugs gave rise to varying degrees of necrosis under the same conditions. Intramuscular injection in the rat was selected for the histologic studies because of the greater homogeneity of the tissue and accuracy in the administration of the drugs. Results were put on a semi-quantitative basis by scoring each muscle according to the type and extent of response. Serial sections were prepared in every instance and were examined throughout by the same pathologist without foreknowledge of the treatments given. All 3 drugs were found to elicit an early inflammatory response characterized by the appearance of a polymorphonuclear infiltrate. In the case of thiomerin this exudate was entirely resorbed within 96 hours without evidence of residual damage. Ninety-six hours after injection of mercuzanthin or mercuhydrin there was evidence of marked fibroblastic proliferation indicating the irreversible character of the initial reaction to these drugs. The relationship of chemical constitution to local toxicity will be discussed.

Comparison of local anesthetic activity of certain aliphatic amines. HERBERT W. TAYLOR, JR. (by invitation), EDWIN J. FELLOWS, EDWARD MACKO (by invitation) and GLENN E. ULLYOT (by invitation). *Research Division, Smith, Kline and French Labys., and Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Penna.* The comparative local anesthetic activity and irritant properties of 32 volatile aliphatic amines have been determined in rabbits by topical application of solutions of the free bases and neutral salts.

Both anesthesia and irritation decreased as the molecular weight was decreased in homologous series and as the point of attachment of the nitrogen was moved toward the center of the carbon chain. The effect of alkylation of the nitrogen or of the carbon chain was irregular. Among these compounds 2-methylaminodecane and 2-amino-2-methyloctane were found to be very active anesthetic agents in concentrations which did not produce irritation.

Effect of tridione (3,3,5-trimethyl oxazolidine-2,4-dione) on the respiration of mouse brain. J. D. TAYLOR (by invitation), R. K. RICHARDS, and G. M. EVERETT (by invitation). *Dept. of Pharmacology, Abbott Research Labys., North Chicago, Ill.* The effect of Tridione on the O_2 uptake of mouse cerebral cortex slices in Krebs-Ringer phosphate solutions containing different substrates was determined by means of the Warburg technique. With glucose, pyruvate, lactate, succinate, acetate, citrate and glutamate as substrates, only glucose showed a significant inhibition with 3.5×10^{-2}M Tridione. At 7×10^{-2}M concentration of Tridione, oxygen consumption was inhibited in all substrates. Inhibition in glucose was the greatest (32%) and acetate the least (17%). In 1.4×10^{-1}M Tridione, inhibition ranged from 62 to 76% depending on the substrate, except for succinate and citrate where inhibition was 33 and 54% respectively. The depression of oxygen uptake by 7×10^{-2}M Tridione in glucose was removed by washing the brain slices after 30 minutes exposure to the drug. The addition of p-phenylenediamine to the inhibited respiring brain slices partially restored the oxygen uptake but glutathione, adenosine triphosphate and creatine were ineffective. Tridione in concentrations up to 7×10^{-2}M did not inhibit anaerobic CO_2 production of mouse brain brei in Krebs-Ringer-bicarbonate. With 1.4×10^{-1}M Tridione there was 26% inhibition. Statistically significant inhibition of O_2 uptake in glucose was also observed in brain slices taken from mice 30 minutes after intraperitoneal injection of 1 or 2 gm/kg. of Tridione. Calculations show that the amounts of Tridione used in these Warburg studies are within the pharmacological range of those used *in vivo*.

Determination of diethylstilbestrol in urine. R. S. TEAGUE, CHRISTIAN WINGARD (by invitation), and ALBERT E. BROWN (by invitation). *Dept. of Pharmacology, Medical College of Alabama, Birmingham, Ala.* A method for the extraction and determination of free and conjugated diethyl-stilbestrol in urine using the Dingemanse antimony pentachloride reaction has been developed. The method consists of the following steps: Urine is acidified and extracted with ether. Free and conjugated diethylstilbestrol are separated by partitioning between ether and aqueous sodium bicarbonate. The free material is then purified by washing the ether solution with aqueous sodium carbonate, extraction with normal sodium hydroxide, neutralization to *p*H 11 with phosphoric acid, and extraction with ether. The ether is evaporated and the residue taken up in ethanol. Aliquots of the ethanol solution are evaporated, the residues dissolved in absolute anhydrous chloroform, antimony pentachloride is added and the density of the resulting color estimated photoelectrically. The bicarbonate solution containing conjugated diethylstilbestrol is acidified with phosphoric acid and extracted with ether. The ether is removed, the residue taken up in decinormal sodium hydroxide, and brought to *p*H 2.8 with phosphoric acid. Hydrolysis is accomplished by autoclaving aliquots of this solution at 180° for 30 minutes. The solutions are then extracted with ether and purified as for free diethylstilbestrol. Recoveries of diethylstilbestrol added to urine have been near 100% and of added diethylstilbestrol glucuronide 90%. In the case of a patient to whom a dose of 100 mg. of crystalline diethylstilbestrol was given orally, the urinary excretion in four days was found to be 8.0 mg. of free diethylstilbestrol, 6.1 mg. conjugated, a total of 14.1 mg.

An attempt to systematize the actions of epinephrine substitutes on smooth muscle organs. C. H. THIENES, JACK LANLEY (by invitation) and WALTER MATTISON (by invitation). *School of Medicine, Univ. of Southern California, Los Angeles, Calif.* A series of compounds chemically related to epinephrine have been tested on isolated small intestine, colon, and uterus of dog, cat, rabbit, rat, and guinea pig and their actions compared with the effect of epinephrine. No consistent relations could be demonstrated, except with arterenol, cobefrine, epinine, metasynephrine, metahydroxy-propadrine, and parahydroxy-propadrine, all of which had actions quite similar to epinephrine on all tissues. The remainder of the compounds were inconsistent in that on some types of organ, or organs of a given animal, the actions were similar to those of epinephrine, on others dissimilar; some compounds were even inconsistent to the extent of causing contraction of an organ at one time and relaxation at another.

Thiocyanate excretion. JESSELENE THOMAS (introduced by MARK NICKERSON). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah.* The use of thiocyanate in hypertensive patients is frequently accompanied by toxic reactions which persist for some time after the drug is discontinued. However, little is known concerning the mechanism of, or methods of promoting, thiocyanate excretion. The fact that thiocyanate and halogen distribution in the body are essentially the same suggested that renal thiocyanate excretion might be related to chloride in the same manner as bromide. Sodium thiocyanate calculated to give extracellular fluid levels of 15 mg.% was injected intravenously into dogs under pentobarbital anesthesia. Diuresis was established by means of water orally, or 5% glucose or 1.8% sodium chloride solution intravenously. Blood and urine samples were collected at intervals and analyzed for thiocyanate and chloride. Sixty thiocyanate clearances provided a range of values from 0.1 to 7.1 cc/minute. Chloride excretion ranged from 0.003 to 1.42 mEq./minute and urine flow from 0.8 to 9.9 cc/minute. Thiocyanate clearances were plotted against chloride excretion for each animal, and a straight-line relationship was obtained in every experiment. Partial coefficients of correlation were found to be +0.92 (P.E. 0.02) for the relationship of thiocyanate clearance to chloride excretion, and only +0.33 for the relationship of thiocyanate clearance to urine flow. It is concluded that renal excretion of thiocyanate is essentially the same as that of chloride. Thiocyanate excretion may be greatly increased by the administration of NaCl.

Cytological effects of alloxan in intact and thymectomized rats. THURLO B. THOMAS (by invitation), PAUL L. EWING and G. A. EMERSON. *Dept. of Zoological Sciences, Carleton College, Northfield, Minn., and Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas.* Thymectomy has been shown to increase the susceptibility of rats to the diabetogenic effects of 0.188–0.25 mM/kg. of alloxan given intravenously; neither thymectomized nor intact rats become hyperglycemic after 0.125 mM/kg. (*Fed. Proc.*, 6: 328, 1947). The cytology of the pancreas, liver, spleen, kidney, lung, adrenal, pituitary, lymph node, thymus and testis has been studied in the above thymectomized and intact rats. The principal cytological effect of alloxan in diabetogenic doses is marked and widespread reduction in nucleoprotein reserves. In thymectomized and intact rats fasted for 24 hr. before and for 12 hr. following alloxan injection, the cytological differences were slight; they involved a somewhat more severe loss of chromatin from the nuclei and ribonucleoprotein

from the cytoplasm of cells in the thymectomized animals. The cytological reaction in both of the above groups of rats was much more severe than in intact animals fasted for 40 hr. before and only 4 hr. after alloxan. Non-diabetogenic doses of alloxan had cytological effects in both intact and thymectomized rats differing only in degree from the above reactions. There appeared to be no direct correlation between the severity of alloxan diabetes, as measured by the blood sugar response, and the extent to which nucleoprotein reserves had been depleted.

Toxic effects of parenterally administered cetylpyridinium chloride. CHARLES R. THOMPSON (by invitation) and HAROLD W. WERNER. *Pharmacology Dept., Research Labys., Wm. S. Merrell Co., Cincinnati, Ohio.* Although quarternary ammonium salts are finding many types of applications clinically there are no data on possible toxic effects of repeated parenteral administrations to laboratory animals. Therefore, the effects of repeated parenteral administrations of one quarternary ammonium salt, cetylpyridinium chloride (Ceepryn Chloride) were investigated in rats and dogs. Daily subcutaneous and intraperitoneal administrations of cetylpyridinium chloride to rats for three weeks in doses of 2.5 mg/kg. decreased the rate of growth. No gross or microscopic tissue alterations were observed which could be attributed to drug administration. The subcutaneous administration of cetylpyridinium chloride to dogs as a 2% aqueous pyrogen-free solution for 1 week in daily doses of 4 mg/kg. caused local abscess formation, hematologic alterations, and toxic changes in the livers and kidneys. Dogs, in which perivascular damage followed the intravenous administration of 0.5% and 2% solutions in daily doses of 1 and 4 mg/kg. for 3 weeks exhibited similar toxic changes. The intravenous perfusion of cetylpyridinium chloride in doses of 4 mg/kg. as a non-irritating 0.1% solution caused no local tissue damage, hematologic alterations, or toxic liver and kidney changes. The systemic toxicity of parenterally administered cetylpyridinium chloride appears to be correlated with the local necrotizing action of the compound.

Comparison of the actions of chloramphenicol and penicillin G against experimental relapsing fever. PAUL E. THOMPSON and MARY C. DUNN (introduced by A. C. BRATTON JR.). *Research Labys., Parke, Davis and Co., Detroit, Mich.* Chloramphenicol (chloromycetin, Parke, Davis & Co.) exhibited marked antispirochetal activity when tested against standardized infections of *Borrelia novyi* in mice (using slight modifications of the methods of Richardson, Walker, Loeb and Miller, *J. Pharmacol.* and *Exp. Therap.* 85 (1): 23, 1945). Evidence of specific activity was indicated by suppression of the blood infection and reduction of mortality among infected animals. Following these observations in preliminary studies, the activity of chloramphenicol was compared on a weight basis with that of crystalline penicillin G in a series of parallel experiments. Chloramphenicol was approximately ¼ as active as penicillin G in suppressing blood infections. However, comparison of the protective action of the drugs against lethal doses of spirochetes revealed that chloramphenicol was approximately 2½ times as active as penicillin G.

Pharmacological evaluation of Trimeton, 1-Phenyl-1-(2-pyridyl)-3-N,N-dimethylpropylamine, and Chlor-Trimeton, 1-(p-Chlorophenyl)-1-(2-pyridyl)-3-N,N-dimethylpropylamine. RICHARD TISLOW, ANNETTE LABELLE, ALOIS J. MAKOVSKY, MARY ANN GRIFFITH REED, MARGARET D. CUNNINGHAM, JANE F. EMELE, ARNOLD GRANDAGE and RUTH J. M. ROGGENHOFER (introduced by H. B. HAAG). *Biological Research Labys., Schering Corporation, Bloomfield, N. J.* Chlortrimeton and Trimeton (*Abstr. 1948 Chicago Meeting, Am. Chem. Soc.*, p. 4K), Pyribenzamine and Benadryl were compared for antihistamine potency (preventing death from 1.1 mg/kg. histamine hydrochloride injected intravenously 1 hour later) and toxicity in guinea pigs. In dogs, Chlor-Trimeton potentiated the epinephrine increase of blood pressure, inhibited the depression of histamine but not of arecoline. Lethal doses of the antihistamine compounds upon intravenous infusion at 25 mg/kg/hour were:

Compound	No. of Dogs	Lethal Dose (mg/Kg)	σ
Trimeton hydrochloride	4	89.0	±12
Chlor-Trimeton maleate	3	97.6	±2
Chlor-Trimeton hydrochloride	3	58.8	±7
Pyribenzamine	4	48.8	±14
Benadryl	2	91.7	±10

Taking the therapeutic index of Pyribenzamine as 1, the value for Trimeton is 4 and Chlor-Trimeton maleate 50. The chlorination of Trimeton in the phenyl ring results in a twenty fold increase in activity without any appreciable change in toxicity. This is in contrast to Benadryl, Pyribenzamine and Thenylene, the halogenation products of which show no such marked increase in activity.

Effect of convulsant and anticonvulsant agents on the activity of carbonic anhydrase. CLARA TORDA and HAROLD G. WOLFF. *New York Hospital and the Depts. of Medicine (Neurology) and Psychiatry, Cornell Univ. Medical College, New York City.* The concentration of carbon dioxide (carbonic acid, bicarbonates) available for metabolic processes in the brain may be an essential factor in the induction and prevention of convulsive seizures according to Lennox and collaborators. The effect of certain convulsant and anticonvulsant

agents on the activity of carbonic anhydrase was investigated to ascertain whether these substances exert any effect on the carbon dioxide uptake and release as effected by this enzyme. The activity of carbonic anhydrase was inhibited in the presence of the convulsant agents used (acetylcholine, camphor, dichloro-diphenyl-trichloro-ethane, digitoxin, pentamethylene tetrazol, picrotoxin, scilliroside, strychnine, and ouabain) in concentrations of 1×10^{-4}M and less, the greatest inhibition being, on the average, 54%. The activity of carbonic anhydrase increased in the presence of the anticonvulsant agents used (hydantoin, methyl-phenyl-ethyl hydantoin, and phenobarbital) in concentrations of 1×10^{-4}M and less, the greatest increase of activity being, on the average, 50%. The results presented suggest that convulsant agents, by inhibiting the activity of carbonic anhydrase (from blood and probably from brain), decrease the removal of metabolic endproducts from brain. Furthermore, anticonvulsant agents, by increasing the activity of carbonic anhydrase (from blood and probably from brain), increase the removal of the above mentioned metabolic products from brain.

Determination of beryllium in biological material. T. Y. Toribara, W. F. Neuman, A. L. Underwood and M. W. Cucci (introduced by H. C. Hodge). *Dept. of Radiation Biology of the Univ. of Rochester, Rochester, N. Y.* Beryllium administered intravenously is very toxic; the LD_{50} is approximately 0.36 mg/kg. Thus for distribution and excretion experiments, not more than 0.2 mg/kg. can be administered. At such a dose level, many tissues may be expected to contain 0.1 microg/g. or less. Thus, any procedure, to be useful, must detect with accuracy much less than one microgram. Chemical methods are not as sensitive as the spectrographic method for the determination of beryllium, but they are adequate for many purposes. Existing methods are too sensitive to *p*H, salt concentration and the presence of other metals. Because of these reasons and also because of the small amounts being sought, the isolation of beryllium is just as important as the determination. This work seeks to improve both the separation and the method of determination. One fluorometric and 5 colorimetric methods of determination and procedures for isolation of small amounts of beryllium will be discussed.

Synergistic effect of sodium acetate and ethanol in antagonizing sodium fluoroacetate (1080) poisoning in mice. W. W. Tourtellotte (by invitation) and J. M. Coon. *Univ. of Chicago Toxicity Laby. and the Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.* Following the suggestive results of Hutchens et al. (*J. Pharmacol.*, in press) Carworth farm mice weighing 18–22 gm. were poisoned with 1080 subcutaneously and immediately treated intraperitoneally with ethanol, sodium acetate, or sodium acetate dissolved in ethanol. The LD_{50} of the 1080 powder used in these experiments was found to be 17.0 mg/kg. The most effective combination of ethanol and sodium acetate found for antagonizing 1080 poisoning was 2.0 gm. (16% solution) to 3.0 gm. (24% solution) of sodium acetate/kg. injected with 1.6 gm. of ethanol (20% by volume)/kg. This saved 90–100% of the mice poisoned with 10 LD_{50} of 1080. When poisoned mice were treated immediately with 1.6 gm/kg. of ethanol or 3.0 gm/kg. of sodium acetate alone, then the LD_{50} of 1080 was raised by a factor of about 3 (54.0 mg/kg.) or a factor of about 4 (63.0 mg/kg.), respectively. However, when poisoned mice were treated immediately with 3.0 gm/kg. of sodium acetate dissolved in 1.6 gm/kg. of ethanol, then the LD_{50} was raised by a factor of 12 (201.0 mg/kg.). These data indicated that ethanol and sodium acetate acted synergistically in antagonizing 1080 poisoning. When treatment was given at 3, 7, 10, and 22 minutes after poisoning, the LD_{50} was raised by factors of approximately 8, 4, 3, 2, respectively. The effect of treating poisoned guinea pigs, rabbit, and dogs with ethanol-acetate solutions and barbiturates will be discussed.

Influence of ethyl chloride spray on deep pain and ischemic contraction of skeletal muscle. J. Travell, and S. H. Rinzler, (by invitation). *Dept. of Pharmacology, Cornell Univ. Medical College, and Cardiovascular Research Unit of Beth Israel Hospital, New York City.* Since painful skeletal muscle spasm may be relieved by ethyl chloride spray, we investigated its effects on deep pain and muscular endurance during ischemia produced by a cuff on the upper arm. Endurance was measured by the number of times the subject could close the fingers during ischemia. A constant rate of 30 isotonic contractions per minute was maintained by a metronome. *1)* After a series of control measurements, one arm was sprayed with ethyl chloride for 5 minutes and endurance determined again on both arms. Tests were run 20 minutes apart; when determined at such intervals on the same day, endurance remains relatively constant. Amounts of ethyl chloride used did not cause frosting, cold pain, or loss of cutaneous sensibility to pinprick. Results indicate that one such application of spray may double muscular endurance during ischemia. *2)* After endurance was determined for 1 arm, without releasing pressure in the cuff, the forearm was similarly sprayed with ethyl chloride for 1 minute and contractions resumed until pain again became intolerable. The procedure was repeated on the other arm, except that a rest period of 1 minute was substituted for spraying. Data show that ethyl chloride spray, applied during ischemia at the limit of endurance,

markedly lessens pain and enables the subject to perform muscular contractions markedly in excess of the increment possible after rest alone. These effects obviously cannot be attributed to circulatory changes in muscle. Similar effects were not secured by one minute immersion of the forearm in cold water (12–20°C.) during ischemia. Results suggest that impulses from superficial structures play an important role in deep pain perception and skeletal muscle function.

Thiouracil administration and thyroidectomy on experimental polyarthritis of rats. HELEN B. TRIPI, WILLIAM C. KUZELL, and GRACE M. GARDNER (introduced by P. J. HANZLIK). *Dept. of Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco 15, Calif.* Albino rats administered 0.1% thiouracil in the diet for periods up to 233 days developed hyperplastic thyroids with nodular hyperplasia. When infected with the L-4 strain of pleuro-pneumonia-like organisms these animals developed a more severe degree of polyarthritis than did the controls, and showed a higher mortality. Thyroidectomized albino rats which had an average basal metabolic rate of only 58% of normal (the same as for the thiouracil-medicated rats) when infected with the same microbes developed an arthritis which was no worse than that in the controls. Other rats poisoned with agents chemically and physically different from thiouracil, namely, arsenic trioxide, isopropyl alcohol and phenobarbital, showed no increase in severity of polyarthritis due to these microbes. Thus, the increased severity of this experimental polyarthritis in thiouracil-medicated rats is believed to be due to some peculiar intrinsic action of thiouracil.

Algesimetry in the study of procaine anesthesia. PAUL E. TULLAR (introduced by THEODORE KOPPANYI). *Dept. of Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.* It was possible to differentiate between the drug factor (D.F.) and the pressure factor (P.F.) in infiltration anesthesia by the combined use of several testing methods. Three types of stimuli (radiant heat, electric and mechanical) were employed to determine the degree, duration, and other features of procaine-infiltration analgesia in man. Pain sensation is measured *a*) in seconds required to produce a sharp burning pain and withdrawal of the finger with a modified Wolff-Hardy stimulator; *b*) in cm. distance between the primary and secondary coils of a Harvard Inductorium; and *c*) in units pressure required to produce sharp 'pricking' pain with a calibrated, spring driven aesthesiometer. At no time were stimuli in excess of 30 seconds for burning pain, 4 cm. for faradic shock, or 10 gm. needle pressure permitted, in order to avoid burn. It was found that stimuli of the above maximal intensities did not interfere with the repeated use of the nail-base areas. After establishing normal responses, test solutions (0.2 to 0.25 cc.) were injected subcutaneously into the sensitive nail-base of eleven subjects for a total of 24 individual tests. The injection of procaine-HCl (1% and 2%) resulted in increased thresholds to all 3 types of stimuli. The anesthesia was of short duration and showed gradual return to normal except in the case of radiant heat where marked hyperalgesia preceded the ultimate return to normal as occurred in a majority of instances. Injection of normal saline resulted in marked anesthesia to mechanical and electrical stimuli, but showed no or little analgesia to radiant heat stimulation.

Changes in blood volume during prolonged pentothal anesthesia. JAMES E. TUREMAN (by invitation) A. H. MALONEY, WALTER M. BOOKER and CLAUDIA M. RATLIFF (by invitation). *Dept. of Pharmacology, College of Medicine, Howard Univ., Washington, D. C.* In connection with our work on prolonged pentothal sodium anesthesia it has been observed that the blood picture follows a more or less definite pattern which tends to represent a hemodilution. An attempt is being made to determine if the hemodilution is real or relative and the possible mechanism. At this time 38 experiments have been completed, using 6 rabbits and 32 mongrel dogs. The dogs were divided into 3 groups, a group on normal diet, a group on high carbohydrate diet, and a group on high protein diet. Initial blood samples were taken, following which dogs were anesthetized with pentothal sodium and maintained in surgical anesthesia for an average of 3 hours. Hemoglobin and/or hematocrit was determined at ½ hour intervals in some instances and in others at 1 hour intervals. Hemoglobin decreased an average of 1.7 gm.% during the first hour then tended to increase until the end of the experiment. The hematocrit decreased an average of 6% during the first hour and increased similarly as the hemoglobin. At the end of the experiments the values approached or equaled the initial values. There was no apparent difference in the groups. In an attempt to discover the mechanism and determine if these results were real or relative, tissue space by the thiocyanate method and plasma volume by the 1824T dye method were determined. In 6 experiments it appears that fluid is drawn from the tissue spaces.

Adrenolytic activity of a number of N-phenyl-isopropyl-beta-haloethylamines. GLENN E. ULLYOT (by invitation), JAMES KERWIN (by invitation), EDWIN J. FELLOWS and EDWARD MACKO (by invitation). *Research Division, Smith, Kline and French Labys., and Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Penna.* Substances with the following structure were compared with 'Dibenamine' for their effect after intravenous administration on the pressor

action of epinephrine in cats anesthetized with pentobarbital sodium: $R—N(R_1)—CH_2—CH_2$ X. Activity comparable with that of 'Dibenamine' was noted when R = phenylisopropyl and R_1 = allyl or isobutyl but where R = allyl or isobutyl but where R × phenylisopropyl and R_1 = ethyl or isopropyl, the compounds were less active. In the case of the derivative in which R_1 = benzyl and R = unsubstituted phenylisopropyl, activity greater than that of 'Dibenamine' was obtained. The same was true when R_1 = benzyl and R = p-methoxy-, p-hydroxy-, 3,4-dimethoxy-, or 3,4-dihydroxyl-phenylisopropyl.

Anticonvulsive properties of myanesin. K. UNNA and A. KAPLAN (by invitation). *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago 12, Ill.* The anticonvulsive effect of myanesin was studied in 500 mice treated with either strychnine or metrazol, or subjected to maximal seizures induced by electroshock. Myanesin was administered subcutaneously in doses ranging from 100 to 500 mg/kg., 10 minutes before the convulsive agent. A dose of 500 mg/kg. represents the paralyzing dose 50. The observation of Berger and Bradley that strychnine convulsions are effectively antagonized was confirmed. Both mortality and severity of the convulsions caused by strychnine (1.75 mg/kg. i.p.) were markedly reduced. The mortality of the mice treated with metrazol (90 mg/kg. i.p.) was less effectively reduced. The metrazol seizure of mice treated with 100 or 200 mg/kg. of myanesin, however, was at least as severe as in the controls and often prolonged. The tonic extensor phase of metrazol convulsions, in which most of the control animals die, was eliminated by myanesin; death of the myanesin-treated mice was delayed. The electroshock (25 mA for 0.3 seconds by eye-electrode) caused typical seizures in 89% and a mortality of 33% of the control animals. None of the myanesin-treated animals died from electroshock. The pattern of the seizures was changed by myanesin (200 mg/kg) in that the tonic flexor-extensor phase was abolished, and only clonic convulsions of a much more violent nature than in the controls were observed.

Elimination of d-tubocurarine in the rat. E. F. VAN MAANEN (introduced by OTTO KRAYER). *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.* Rats were anesthetized with urethane. Contractions of the gastrocnemius muscle, maximally stimulated through the sciatic nerve, were recorded. The total curarizing dose was measured at different rates of administration of d-tubocurarine. The amount of fluid injected per hour was 4.78 cc. It was found that, when d-tubocurarine was administered at a rate of 1000 γ/hr/kg. rat or more, approximately 70 γ/kg. rat was necessary for complete curarization of the gastrocnemius muscle. Decreasing the rate of administration resulted in an increase in the amount of d-tubocurarine necessary for total curarization. It was impossible to obtain complete curarization, when less than 80 γ/hr/kg rat was administered, even though the injection lasted for 5 hours or more. In nephrectomized rats the total curarizing dose differed very little from that of normal rats, when 150 γ/hr/kg rat or more was administered. However, when less than 150 γ/hr/kg rat was injected the total curarizing dose was less than that of normal rats. Complete curarization was obtained in nephrectomized rats when the rate of administration was as low as 60 γ/hr/kg. rat.

Secretion of cortical hormone by the isolated adrenal. MARTHE VOGT (introduced by H. B. VAN DYKE). *Pharmacological Laby., Univ. New Buildings, Edinburgh, Scotland.* The isolated adrenal of a dog, perfused with blood by means of a Dale-Schuster pump, secretes cortical hormone in quantities detectable by biological assay on adrenalectomized rats exposed to cold. The rate of secretion is not much lower than that of the gland *in situ* during the course of an abdominal operation. It is not abolished by perfusing with blood from a hypophysectomized donor, and is immediately enhanced by the administration of corticotrophic hormone. The rate of secretion is unaffected by a number of metabolites (glucose, lactic acid, amino acids), but is augmented by adenosine-tri-phosphate and by creatine phosphate. Inorganic phosphate has no such action. These effects may be connected with the energy requirements of the synthetic processes continually going on in the gland. Another factor found to increase the output of cortical hormone is a raised concentration of potassium in the plasma. In order to obtain an acceleration of cortical secretion which can be detected by the assay employed, the plasma potassium has to be increased considerably, i.e., to values which may occur in adrenalectomized dogs, but not in normal animals. It is interesting that this effect is obtained in the absence of the pituitary, and it would be desirable to know whether smaller rises in the rate of secretion, such as cannot at present be established by direct biological assay of the hormone in blood, would result from more physiological variation in the potassium level of the plasma.

Therapeutic efficiency of various dosage schedules of oxophenarsine in experimental trypanosomiasis. HARRY A. WALKER, SUSANNE WILSON (by invitation), and A. P. RICHARDSON. *Dept. of Pharmacology, Emory Univ. School of Medicine, Emory Univ., Georgia.* Recently, the relation of dosage schedule to the therapeutic effect of a number of chemotherapeutic agents in the treatment of experimental bacterial and protozoal infections

has been investigated in order to establish optimal dosage regimes. This report concerns the relative therapeutic efficiency of dosages of oxophenarsine given by single and multiple intraperitoneal injections against *Trypanosoma equiperdum* infections in the mouse. Previously it has been demonstrated with this infection in the rat that arsenoxide (oxophenarsine) produces a prompt trypanocidal effect as indicated by a decrease in parasite density of the peripheral blood within a period of 30 minutes. In our experiments mice were infected intraperitoneally with an inoculum of 100 million parasites/kg. of body weight and remained untreated until 24 hours after inoculation. From stained thin smears made every 5 minutes after a single intraperitoneal injection of oxophenarsine it was found that within 10 minutes a decrease in parasite density occurred. Within 45–60 minutes the maximum effect was obtained. On the basis of the above data and the fact that the arsenic content of the blood after an intravenous injection of oxophenarsine falls abruptly to a low level by the end of 1 hour, the ED_{50} of oxophenarsine given intraperitoneally in 10 equal doses every 1.5 hours starting 24 hours after inoculation was determined and found to be 0.30 mg/kg. The ED_{50} of a single dose injected 24 hours after inoculation was 0.15 mg/kg. The data indicate that a single dose of oxophenarsine exerts a significantly greater therapeutic effect than that produced by the same dosage given in 10 equal doses at intervals of 1.5 hours.

Cardiac and calorigenic actions of dicumarol and dinitrophenol. R. P. WALTON, C. B. HANNA (by invitation), R. P. PRYSTOWSKY (by invitation), and J. S. LEARY (by invitation). *Dept. of Pharmacology, Medical College of South Carolina, Charleston, S. C.* Calorigenic and cardiac actions of dicumarol at high dose levels have been described respectively by Wakim, Chen and Gatch (*S. G. & O.* 76: 323, 1943) and Carlson and Seager (*Fed. Proc.* 7: 210, 1948). The experiments reported here were aimed at defining the nature of the cardiac action. The contractile force of a section of the right ventricle was determined directly in open-chest dog preparations along with other observations of systemic changes. Dicumarol was administered in doses of 10 to 100 m.p.k. by i.v. infusion over periods of 30 min. Doses at the low extreme had no clear effect while those at the high extreme quickly produced terminal fibrillation with little evidence of contractile force stimulation. Intermediate doses in about 60% of the trials produced first slight to moderate depression followed by distinct to marked increases in contractile force, stroke amplitude, heart rate, arterial pressure, external respiratory movements and rectal temperature. The contractile force increases usually reached a maximum about 60 min. after the start of the infusion. For the most part, effects developed at parallel rates. In a few instances, however, the cardiotonic action clearly preceded the temperature rise and also was characteristically obtained in experiments in which the rectal temperature rise was prevented by the use of ice packs. Moderate increases in venous pressure were considered insufficient to account for all of the increases in contractile force. A closely similar group of responses were obtained with dinitrophenol in the dose range of 10 to 20 m.p.k. Distinct contractile force increases of the same order were obtained by the application of diathermy. Perfusion of isolated rabbit hearts with dicumarol or dinitrophenol produced marked depressant effects preceded, at times, by stimulation. In the open-chest experiments and in intact dogs receiving 250 to 400 m.p.k. dicumarol orally ECG records commonly showed T-wave reversal and deflection of the RS-T segment but did not usually show A-V dissociation until near the terminal stages.

Effect of various lactones and of veratrine on the mammalian heart. R. P. WALTON, M. DEV. COTTEN (by invitation), and O. J. BRODIE (by invitation). *Dept. of Pharmacology, Medical College of South Carolina, Charleston, S. C.* Contractile force changes of a section of the right ventricle were determined in open-chest vagotomized dogs along with ECG and arterial pressure recordings. Four lactones, previously shown to have digitalis-like effects on the cold-blooded heart, had no stimulant effects on the mammalian heart *in situ*. Administered by i.v. infusion over periods of 30 min., the compounds were: psi-santonin, 30 to 150 m.p.k.; 1-ascorbic acid, 50 to 250 m.p.k.; beta-gamma-angelica lactone, 400 to 800 m.p.k.; di-lactone of pulvinic acid, saturated solution in saline, 20 cc/kg. The first 3 of these compounds produced significant depression suggestive of that obtained with alcohol. The di-lactone of pulvinic acid had no clear effect; doses up to 2 m.p.k. were injected in dioxane solution with, however, immediate precipitation.—The commercial *N.F. V* mixture of alkaloids termed 'veratrine' was administered by infusion as above; also, by immediate injections, doses were alternated with epinephrine both before and after dibenamine. Pressor effects and cardiac stimulation with veratrine and epinephrine were generally similar although the effects of veratrine were more sustained. Dibenamine blocked effects of both drugs in a parallel manner. In contrast to epinephrine, veratrine, in small doses frequently produced temporary depression and, in larger doses, a greater tendency to fibrillation; also, stimulant effects more frequently diminished on repeated injection. Veratrine doses of 350 gamma/kg. corresponded approximately to epinephrine doses of 5 gamma/kg. Cardiac stimulant effects of veratrine were accompanied by strong skeletal

muscle movements which were disturbing if not blocked by curare. ECG effects consisted mainly in the R and S-wave voltage changes which usually occur with left axis deviation, frequent reversal of T-wave and, in the terminal stages, ventricular ectopic beats with frequently a bi-directional rhythm. A-V dissociation did not commonly occur until near the terminal stages. In isolated rabbit heart preparations, veratrine concentrations 10 to 20 times those of epinephrine produced generally similar effects and could be successively repeated several times.

Pharmacological studies on 2- [N-p'-tolyl-N-(m'-hydroxyphenyl)-aminomethyl-] imidazoline hydrochloride (C-7337). M. R. WARREN, R. A. WOODBURY and J. H. TRAPOLD (by invitation). *Division of Pharmacology, Univ. of Tennessee, Memphis, Tenn.* 2-[N-p'-tolyl-N-(m'-hydroxyphenyl)-aminomethyl-] imidazoline hydrochloride (C-7337) has been shown to be an effective adrenergic blocking agent in dogs following oral and intravenous administration. Its onset of action is more rapid than that reported for Dibenamine after intravenous injection but its duration is shorter. The pressor response elicited by anoxia or the intravenous injection of carbaminoylcholine in atropinized dogs is converted into a depressor response. Doses which cause 'epinephrine reversal' have no significant effect on the depressor action of acetylcholine and histamine. Ephedrine sulfate given intravenously will combat the hypotensive action induced by rapid injection of C-7337 and decreases the duration of its adrenolytic effect. Isolated uteri from estrogen-treated rabbits are stimulated by weak concentrations but only inhibition is obtained with greater concentrations. Contractions elicited by histamine, epinephrine, pitocin and pitressin are likewise inhibited. Spontaneous contractions of the isolated ileum of the rabbit, cat and rat are suppressed as are contractions induced by barium chloride, acetylcholine, and pitressin. This inhibitory action on the intestine is not abolished by atropine. Acute toxicity work in dogs indicates that approximately 100 mg/kg., administered twice daily, are required to kill 50% of the animals within a period of 1 week. Single doses as large as 500 mg/kg. failed to prove lethal. Chronic oral administration of C-7337 in doses up to 50–60 mg/kg. twice daily for three months caused no appreciable gross toxic manifestations with the possible exception in young or pregnant animals.

Isomers of methadon and isomethadon and respiration of brain homogenates. DANIEL T. WATTS, (introduced by C. L. GEMMILL). *Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville, Va.* The effect of d-, l-, and d,l-methadon and d-, l-, and d,l-isomethadon on the oxidation of glucose, succinate and ascorbate by fortified brain homogenates was investigated. A Warburg apparatus was used to determine simultaneously in triplicate the oxygen uptake in control vessels and in vessels containing the d-, l- and d,l-methadon or isomethadon. Oxygen uptake, expressed as percentage inhibition (O_2 uptake in control vessels, minus uptake in vessels with drug, divided by control uptake) was as follows:

Substrate	Drug Conc. Molar	Methadon			Isomethadon		
		d,l-	d-	l-	d,l-	d-	l-
Glucose	0.005	65	50	56	76	55	62
Succinate	0.003	—	—	—	57	57	60
Succinate	0.007	74	66	76	—	—	—
Ascorbate	0.005	53	53	76	84	82	86

The observed decrease in O_2 uptake may be due to a general enzymatic inhibition or to a specific block at the cytochrome-cytochrome oxidase level. Inhibition of ascorbate oxidation which requires only the catalytic action of cytochrome-cytochrome oxidase indicates blockage occurs at this level.

Effect of local anesthetics on respiration of brain homogenates. DANIEL T. WATTS, (introduced by C. L. GEMMILL), *Dept. of Pharmacology, Univ. of Virginia, Medical School, Charlottesville, Va.* The effect of selected local anesthetics on the oxidation of glucose, succinate and ascorbate was investigated. The homogenates were diluted to reduce endogenous activity to a minimum and then reinforced with as many factors as possible to obtain maximum oxygen uptake for the substrate under investigation. Additional cytochrome c, calcium and aluminum ions were added for succinate and ascorbate oxidation. Hexose diphosphate, adenosine triphosphate, diphosphopyridine nucleotide, cytochrome c, nicotinamide and magnesium ions were added for glucose oxidation. Oxygen uptake was measured with Warburg manometers simultaneously in duplicate for controls and for vessels containing the drugs at a final concentration of 0.005 M. Oxygen uptake expressed as per cent inhibition was as follows:

	Glucose	Succinate	Ascorbate
Cocaine HCl	32	9	0
Procaine HCl	22	11	9
Metycaine HCl	46	8	4
Pontocaine HCl	64	44	36
Nupercaine HCl	74	95	68

In a further attempt to isolate the point of blockage by the local anesthetics it was found that nupercaine *a*) did not inhibit the reduction of methylene blue by succinate and homogenate; *b*) inhibited the oxidation of reduced cytochrome by homogenate in presence of oxygen; *c*) inhibited the reduction of oxidized cytochrome c by homogenate and succinate when the cytochrome oxidase

was inhibited by cyanide. These observations indicated that the cytochrome oxidase and the factors necessary for the reduction of cytochrome c are sensitive to the action of nupercaine.

Cardiac-active principles in osage orange. R. A. WAUD, C. W. GOWDEY (by invitation) and J. S. LOYNES (by invitation). *Dept. of Pharmacology Univ. of Western Ontario, London, Canada.* It has been shown previously by one of us (RAW) that Osage Orange contains a substance or substances which cause marked augmentation of the isolated hypodynamic frog's heart when perfused through the vena cava. Prolonged augmentation was also shown in a large percentage of isolated rabbit hearts perfused by the method of Langendorff. The augmentation was not accompanied by changes in heart rate nor by changes in conduction. Systolic standstill did not occur. These principles have been investigated further and we believe that a large part of the cardiac activity is due to a phytosterol which occurs in the non saponifiable fraction of the oil contained in the Osage Orange. This sterol has been isolated and obtained in crystalline form. Definite activity is shown by a concentration of 1:200,000 of this crystalline substance. From the work of Danilewsky in 1907 on the actions of cholesterol on the frog's heart one might expect the results we have described because of the close structural relationship's between the plant sterols and cholesterol. We have repeated the work with cholesterol and in addition have tested the activity of several other sterols. Ergosterol has a similar but weaker action. Similar activity was found in the California Orange.

Absorption and distribution of isopropyl alcohol. JOAN WAX (by invitation), FRED W. ELLIS, and ARNOLD J. LEHMAN. *Dept. of Pharmacology, School of Medicine, Univ. of North Carolina, Chapel Hill, N. C.* As part of a systematic pharmacologic study of isopropyl alcohol (IPA) the gastro-intestinal absorption and tissue distribution of this substance was investigated. In pentobarbitalized dogs *in situ* stomach and/or intestinal loops were isolated, and IPA absorption was studied as affected by site and extent of absorbing surface, concentration, time, successive doses, and systemic ethyl alcohol. Absorption of IPA occurred from all portions of the digestive tract, most rapidly in the jejunum and least in the stomach. During a 30-minute absorption period IPA was distributed to the spinal fluid, brain, liver, kidney and skeletal muscle. Spinal fluid and blood concentrations were approximately equal, but there was no constant relationship between tissue and blood concentrations. Increased absorption area resulted in increased tissue concentration, but did not influence total absorption. The percentage absorption was not markedly affected by the concentration of IPA although at the site of highest absorption (jejunum), 10% strength was absorbed significantly better than 5%, 50%, and 99%. Previous absorption of IPA from intestinal loops did not influence subsequent absorption from adjacent loops. Ethyl alcohol administered intravenously markedly decreased the intestinal absorption of IPA.

Fate of certain antihistamines. E. LEONG WAY, ROBERT E. DAILEY (by invitation) and DONALD L. HOWIE (by invitation). *Dept. of Pharmacology, The George Washington Univ. School of Medicine, Washington, D. C.* Certain antihistaminics have been successfully determined in biologic tissue by application of the Brodie methyl orange technique. A high degree of specificity was obtained when the method was modified for determining tripelennamine (pyribenzamine) and phenindamine (thephorin). Tripelennamine was used as the standard for comparison with other agents. In rats fasted for at least 18 hours, after a single oral dose of 100 mg/kg. about 35% of the total dose was recovered in the gastro-intestinal tract after 2 hours and about 5% after 4 hours. After intraperitoneal administration of tripelennamine to rats or guinea pigs high concentrations were obtained in the spleen, lung, liver and kidney. Concentrations in the brain were almost as high and low levels were found in muscle, heart and blood. Preliminary results with phenindamine indicate that its distribution follows somewhat the same pattern as that for tripelennamine. After 3 daily doses of either tripelennamine or phenindamine for several days, slight or no levels were detected in the tissues after 24 hours, indicating little or no storage of the compounds. Evidence that both compounds are rapidly metabolized is indicated by the fact that only slight amounts of either compound were recovered in excreta after parenteral administration. Preliminary *in vitro* results on tripelennamine indicate that the detoxication site is the liver.

Fate of dl-methadon. E. LEONG WAY, CHEN-YÜ SUNG (by invitation) and WILLIAM P. MCKELWAY (by invitation). *Dept. of Pharmacology, The George Washington Univ. School of Medicine, Washington, D. C.* The fate of dl-methadon was investigated in rats by application of Brodie's methyl orange technique. The method was modified to give a high degree of specificity for methadon in biological tissues. In fasted rats, about 25% of the total methadon dose given orally was recovered in the gastro-intestinal tract after 2 hours. The compound was found to be firmly bound by all tissues, especially muscle. After intraperitoneal administration of methadon highest concentrations were found in the lung, liver, kidney and spleen. Low levels were present in blood, brain, muscle and heart. After injections of methadon 2 or 3 times daily for several days, there was little

or no methadon in tissues removed 24 hours after the last dose, indicating that prolonged storage of methadon does not occur. Only a small fraction of the total administered dose was recovered in the urine as methyl orange reactant. It was also found that in vitro, liver and kidney slices metabolized methadon within 4 hours to substances which are not measured by the methyl orange technique.

Vasoconstrictor activity of epinephrine, ephedrine and a new sympathomimetic diamine 1-(m-hydroxyphenyl-N²-methylethylene diamine (NU-1683). HOWARD WAYNE (by invitation), LEWIS FRANKLIN (by invitation), and HAROLD D. GREEN. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.* Lehmann and Randall recently described a series of sympathomimetic diamines (*J. Pharm. and Exp. Therap.* 93: 114, 1948). We compared the vasoconstrictor activity of one of these, NU-1683, with that of epinephrine and ephedrine by injections of each into the femoral artery of dogs while measuring the venous outflow with a modified Gaddum-type meter connected to the femoral vein. 1 μgm of epinephrine reduced flow by 15–78% (av. 49%) in 35 injections in 6 dogs. Latency of response was 20–30 seconds, minimum flow occurred approximately 50 seconds later and lasted 10–50 seconds. Duration of response was 2.8 min. (range 1.3–6.7 min.). Tachyphylaxis did not occur. 1–3 mg. of NU-1683 reduced flow 24–78% (av. 45%). Minimum flow lasted 20 seconds, duration of response was 4.2 minutes (range 1.3–8.5 mins.) in 13 injections in 7 experiments. Tachyphylaxis did not occur. 1 mg. of ephedrine reduced flow 25% (range 15–43%) in 6 injections in 3 experiments. Minimum flow lasted 13 seconds. Duration of response was 0.9 minutes (range 0.5–1.2 mins.). Tachyphylaxis did occur. With each drug the latent period of response and times for occurrence of minimum flow were about the same. Sufficient data is not yet available to predict the dose response curves for these drugs. A detectable reduction of flow (10%) is usually produced by 0.1/gm. of epinephrine, 0.1 mg. of NU-1683 and 0.1 mg. of ephedrine, and an almost complete cessation of flow (90% reduction) by 3–10 gm. of epinephrine and 10 mg. of NU-1683.

Separation by fluoroacetate of energy sources for smooth muscle contraction. JAMES R. WEEKS (by invitation) and MAYNARD B. CHENOWETH. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, Mich.* Contraction of rabbit jejunum segments in oxygenated Krebs-Henseleit solution is maintained equally well by 0.005 M glucose or sodium acetate. Addition of sodium fluoroacetate (FA) decreases contraction amplitude. Communication with Dr. Alfred Farah during early phases of our study disclosed that muscles contracting in glucose are more resistant to FA than in sodium acetate. This has also been found true for acetate in the form of monoacetin, although this is the only effective *in vivo* FA antagonist. By use of various concentrations of FA acting on jejunal segments in 0.005 M glucose it has been found that even at very high concentrations of FA reduction of contraction amplitude below about 35% of initial amplitude does not occur. In 0.005 M acetate FA in similar high concentrations reduces contraction almost to zero. Addition of glucose to 0.005 M returns contraction to the approximate level expected had glucose been present initially. Yet at low concentrations of FA acetate is a better antagonist than glucose. These findings may be harmonized by presuming that intestinal muscle obtains energy aerobically from breakdown of glucose to pyruvate and subsequently from syntheses in the tricarboxylic acid cycle involving acetate. When acetate as the sole available substrate is blocked from entry into the cycle by high concentrations of FA, contraction ceases; while low concentrations of FA are unable to produce a block in presence of excess acetate. In presence of glucose energy for partial contraction is available despite presence of FA block of acetate.

Mechanism of cardiac injury in experimental hypothermia. DAVID WEINER, KURT LANGE and MICHAEL M. A. GOLD (introduced by M. G. MULINOS). *Dept. of Medicine, New York Medical College, New York City.* Rabbits suffering from lowering of body temperature show a reduction in heart rate directly proportional to the fall in body temperature. The P-R interval and the QRS complex show a prolongation roughly proportional to the fall in body temperature. The relative duration of electrical systole as expressed by Bazett's formula reveals a prolongation which becomes relatively greater as body temperature decreases. The very marked S-T segment and T wave changes under such conditions show individual differences in extent and localization in both rabbits and dogs. The changes in rate and conduction are exclusively the result of the direct effect of cold. The prolongation of electrical systole is partly the result of cold directly on the muscle fibres and partly the result of anoxia due to lowered oxygen dissociation. The T wave changes are exclusively the result of anoxia. The anoxic nature of the S-T segment and T wave changes as well as part of the prolongation of electrical systole is proved by the fact that increasing the oxygen dissociation of the blood by acidification reverses them to normal. Acidification of the blood of uncolled rabbits and dogs does not change the EKG. Anoxemia plays no role in the production of any of the changes seen in the heart with exposure to cold. We are dealing with anoxia without anoxemia.

Fate of dicoumarol in man. MURRAY WEINER

(by invitation), JULIUS AXELROD (by invitation), SHEPARD SHAPIRO (by invitation) and BERNARD B. BRODIE. *Depts. of Biochemistry and Medicine, New York Univ. College of Medicine, and New York Univ. Medical and Research Services, Goldwater Memorial Hospital, New York, N. Y.* A method for the estimation of dicoumarol in biological material has been devised. It is specific in that neither normal biological material nor metabolites of the drug are included in the measurement. With this method, those factors which affect the plasma concentration of the drug such as absorption, excretion, metabolism and tissue localization are being studied. Only traces of dicoumarol were found in the urine after its administration, indicating the almost complete metabolism of the drug in the body. The rate of metabolism is slow and varies with the dose. It varies from about 25% per day at the upper therapeutic plasma levels to over 75% per day at low plasma levels. There are wide variations in the rate of transformation in different individuals, even at similar plasma levels. The drug is highly localized on plasma proteins (about 99% plasma binding) and in various tissues. Its low concentration in plasma water explains in part, its low transformation rate. The rate of absorption of the drug from the gastrointestinal tract is slow and varies with the individual and with the dose. Thus in the same individual a dose of one mg/kilo yielded a peak plasma concentration within 6 hours while a dose of 8 mg/kilo did not yield a peak level until more than 24 hours. The relationship between dicoumarol concentration and prothrombin time elevation is being studied in the hope that safer dosage with the drug may be achieved by following plasma levels.

Influence of xanthopterin on the folic acid content of liver, *in vitro*. ARNOLD D. WELCH, EVELYN M. NELSON (by invitation) and MARILYN F. WILSON (by invitation). *Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland,* The activity for *L. casei* or *S. faecalis* of rat liver homogenate or cell-free extract is increased by incubation at *p*H 7, 37°C.; if added promptly, the increase is augmented by xanthopterin (X). The additional material formed behaves like pteroylglutamic acid (PGA); added PGA disappears but little, if at all. Homogenate from PGA-deficient rats is unaffected by X. The PGA content of pig liver homogenate also is increased by X; in a typical 18-hour incubation, the PGA content (in μgm/gm. of liver) rose, at *p*H 7, from 0.25 to 3.0 (4-hour figures somewhat lower); at *p*H 4.7, 0.65 without or with X; at pH 7 (4 hours) followed by *p*H 4.7 (14 hours), the values rose from 1.7 to 18.5. At *p*H 7, added pteroylhexaglutamylglutamate (PG_7) is partially converted to PGA, at *p*H 4.7 nearly completely; the conversion is unaffected by X. Apparently, the 'potential PGA' of pig liver is resistant, at *p*H 4.7, to enzymic conversion to PGA; however, at *p*H 7 this form is converted enzymatically to a compound, presumably PG_7, that is susceptible to enzymic conversion to PGA, particularly at *p*H 4.7. Conceivably, the precursor of PG_7, at *p*H 7, is subjected to two reactions, a slower one leading to formation of PG_7, the other rapidly yielding an inactive substance; X may inhibit this second reaction. Although X also may serve as a precursor, of PGA (through synthesis of PG_7 or its precursor), there is now but little evidence for this (no aug. mentation by reduced DPN, ATP, p-aminobenzoylglutamate or its N-formyl derivative). Experiments with isotopically labeled compounds will be described.

Pharmacology of a new cholinergic agent. W. CLARKE WESCOE (by invitation) and WALTER F. RIKER. *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* The compound, 3-acetoxy phenoxy trimethylammonium methylsulphate (Nu 2017, Hoffmann-LaRoche, Inc.), is of considerable pharmacologic and academic interest because of its close chemical relationship to acetylcholine and neostigmine. It possesses both nicotinic and muscarinic properties; injected into the intact cat it produces all the characteristic signs of acetylcholine action. Compared with acetylcholine its duration of action is longer, though still brief, and its potency is less. Like acetylcholine it is inactivated rapidly by the specific and nonspecific cholinesterases of erythrocytes, brain, and serum; its affinity for the specific type esterase is lower than that of acetylcholine. It possesses the immediate and intense effects of neostigmine on skeletal muscle, providing thereby additional evidence for the direct action of neostigmine on this effector.

Pentobarbital tolerance in rabbits. B. A. WESTFALL (introduced by J. K. W. FERGUSON). *Dept. of Physiology and Pharmacology, Medical School, Univ. of Missouri, Columbia, Mo.* Seventeen young albino rabbits (mean weight 2.31 kg.), selected from a pool of several litters were injected intravenously with an anesthetic dose of pentobarbital sodium (40 mg/kg.), and the sleeping-time noted for each animal. One week later the same 17 animals were injected with the same dose of pentobarbital sodium (40 mg/kg.), the sleeping-time noted, and this routine was continued at weekly intervals until a total of five injections had been given. The data reveal a significant difference (about 30%) between the mean sleeping-time (121.1 min.) after the initial dose as compared to the mean sleeping time (88.6 min.) of the same animals after the same dose of pentobarbital one week later. However, the latter value (mean, 88.6 min.) was almost identical with the mean sleeping time values observed after the third (mean, 87.7 min.), fourth (mean, 87.6 min.) and fifth (87.9 min.) injections

of pentobarbital. It might be pointed out that this study is the first reported in which the repeated injections of pentobarbital were as infrequent as one week apart and that the tolerance developed as a result of the initial dose, not only persisted at least a week, but was maintained by weekly injections of the pentobarbital.

Potentiation by means of CO_2 of the action of penicillin against pneumococcus infection. Richard W. Whitehead, J. Howard Williamson (by invitation), William E. Clapper (by invitation), Joseph N. Spencer (by invitation) and William B. Draper. *Depts. of Physiology and Pharmacology, and Bacteriology, Univ. of Colorado Medical Center, Denver, Colo. In vitro* experiments have shown penicillin to be most effective as an antibiotic in an acid medium. It was thought to be of interest to determine the action *in vivo* of induced carbon dioxide acidosis upon the effectiveness of penicillin using white mice as experimental animals. Type III pneumococcus was used as the test organism. One thousand lethal doses were given as the standard quantity for a 20-gm. mouse. Preliminary studies showed that the maximum protection with a single dose of penicillin was obtained with 2500 u. Acidosis was induced and maintained for 45 minutes by inhalation of 20% CO_2. In control experiments it was shown that CO_2 alone had no effect upon the time of death of the animals infected with pneumococci. A series of 9 experiments was performed using a total of 72 animals, all of which were given 2500 u of potassium penicillin G at the time of inoculation. Thirty-six of these animals were treated for 45 minutes with CO_2 2 hours after having been inoculated with the test organism and penicillin. The average life span for the animals treated with penicillin alone was 38.9 hours. The average survival time for the animals given penicillin and carbon dioxide was 47.1 hours. The difference of 8.2 hours in survival time was shown to be statistically significant.

Biopsy technic and analysis of variance applied to pentobarbital inhibition of brain oxidations. D. S. Wilkins (by invitation), R. M. Featherstone, C. E. Gray (by invitation), J. T. Schwidde (by invitation), and M. Brotman (by invitation). *Divisions of Anesthesiology and Neurosurgery, Depts. of Surgery and Pharmacology, College of Medicine, State Univ. of Iowa, Iowa City, Ia.* This experiment combines a method of dog brain biopsy with analysis of variance and standard manometric technic to ascertain a suitable pentobarbital concentration for studying that drug's effect on the oxygen consumption of various brain levels. The method allows dog brain tissue to be its own control in either *in vitro* or *in vivo* experiments upon drug effects. The dog's calvarium is removed under general anesthesia. Six days later the brain is biopsied, using procaine in the scalp wound, and tissue slices uninfluenced by drugs are prepared. An attempt is here made to establish a pentobarbital concentration which significantly depresses cortical oxidations without completely inhibiting them. Cortex from each of 5 dogs was treated with *1*) 0.00, *2*) 0.04, *3*) 0.08 and *4*) 0.12% pentobarbital. Glucose was added at 90 minutes. Small sample statistical analysis of variance (Snedecor, *Statistical Methods*, 1946) was applied. Main variations were dogs, treatments, time.

Variation Source	Degrees Freedom	F	Significant at 1% Level
Dogs	4	2.916	4.37
R_x (1) and (2)	1	68.681	8.02
(2) and (3)	1	11.849	8.02
(3) and (4)	1	0.000	8.02

(Error term: first order interaction, making summary applicable to general dog population).

Q_{O_2} d.w.	(1)	(2)	(3)	(4)
60–90 min.	0.183	0.072	0.024	0.029
120–150 min.	0.141	0.046	0.021	0.021

Pentobarbital 0.04% appears more satisfactory for the continuance of this study than 0.12% (Quastel) or 0.08% which gave essentially complete inhibition.

Immediate reactions of mice to large amounts of ouabain. W. Lane Williams and Robert Tarail (introduced by R. N. Bieter). *Depts. of Anatomy and Medicine, Univ. of Minnesota, Minneapolis, Minn.* Mice (20–25 gm.) tolerate as much as 2 mg. of ouabain (subcutaneous or intraperitoneal) provided the glycoside is administered in several unit amounts of not more than 0.25 mg. during an interval of 30–60 minutes. Such daily treatment can be continued for a considerable period (*Federation Proc.* 7: 259, 1948). Mice thus treated eventually showed punctate myocardial necrosis and renal and hepatic lesions. Mice also tolerated simultaneous treatment with relatively large amounts of ouabain and calcium provided the total dose for the interval did not exceed that mentioned above. However, a majority of mice receiving a single injection of more than 0.4 mg. of ouabain died within 15 to 28 minutes and showed massive pulmonary edema and hemorrhage. Pulmonary involvement was more extensive than that observed terminally in mice treated chronically with similar amounts of ouabain but in divided doses. Some of these mice received as much as 2 cc. of saline as solvent or vehicle for the glycoside (Lilly preparation of Strophanthus gratus). Equivalent amounts of saline were well tolerated in daily single unit injections for 30 days. The lesions indicate that the acute effects of large amounts of ouabain are directly or indirectly pulmonary. Upon relatively long-term treatment

similar amounts (as to total daily dose) administered as several injections during 30–60 minutes usually produced myocardial damage without concomitant immediately lethal pulmonary lesions.

Respiratory activity in vitro of cardiac muscle of ouabainized dogs. ALBERT WOLLENBERGER (introduced by OTTO KRAYER). *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.* The cardiac glycoside ouabain was administered to dogs by slow continuous intravenous infusion. The heart was excised at the onset of cardiac irregularities or of ventricular fibrillation. Slices of left ventricular muscle were prepared and incubated in glucose-phosphate-saline. During the first 30–60 minutes these slices respired at a somewhat higher rate than cardiac slices of control dogs. Subsequently, however, the respiratory rate declined well below the control level, particularly in the case of the slices of fibrillating hearts. Extent and rapidity of the decline were inversely proportional to the tissue concentration. At a tissue concentration of 1% or less, the slices of fibrillating hearts ceased to respire during the second hour of incubation.

Toxicity of hydroquinone for laboratory animals. GEOFFREY WOODARD, E. CONWAY HAGAN, and JACK L. RADOMSKI (introduced by BERT J. VOS). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Because of the interest in the proposed use of hydroquinone as an antioxidant for edible fats, experiments were undertaken to determine its toxicological properties as well as those of its oxidation products quinhydrone and quinone. Approximate acute oral LD_{50}'s in mg/kg. of hydro-quinone in 2% aqueous solutions are: rats, 320; mice, 400; guinea pigs, 550; pigeons, 300; cats, 70; dogs, 200; and in rats for quinhydrone, 225; and quinone, 130. The approximate intravenous LD_{50}'s in rats are: hydroquinone, 115; quinhydrone, 35; and quinone, 25. The acute toxicity of hydroquinone is reduced in the non-fasted rat and is influenced by the concentration administered. Symptoms of hydroquinone poisoning develop 30 to 90 minutes after oral administration and consist of hyperexcitability, tremors, convulsions and, in addition, salivation in dogs and cats, emesis in dogs and pigeons, and incoordination of the hind limbs of dogs. Deaths occur within a few hours. Quinhydrone and quinone, however, do not produce the same symptoms of poisoning and deaths may be delayed several days. Oral administration of hydroquinone at 100 mg/kg. in the dog and at 70 mg/kg. in the cat produces mild to severe swelling of the area around the eye, of the nictitating membrane, and of the upper lip. However, dogs receiving 25 and 50 mg/kg. daily for 4 months have shown only slight eye involvement. Routine blood counts in cats and dogs acutely and subacutely poisoned with hydroquinone indicate increased activity of the cell-forming tissues.

Evaluation of drugs possessing anti-dysmenorrhoea properties. R. A. WOODBURY and PAUL P. C. FENG (by invitation). *Division of Pharmacology, Univ. of Tennessee, Memphis, Tenn.* Vasopressin (0.05 to 0.3 vasopressor units of Pitressin) injected intravenously in humans immediately caused marked uterine activity and caused or intensified the distress characteristically like that of dysmenorrhoea (*Federation Proc.* 5: 215, 1946). These observations suggest the hypothesis that drugs which are effective in relieving dysmenorrhoea symptoms by mechanisms other than through analgesia may well possess anti-vasopressin activity to one or more of the actions of vasopressin. Drugs useful in the treatment of dysmenorrhea such as atropine, papaverine, Pavatrine, Trasentin, Nethamine, Isopropylepinephrine, neostigmine, progesterone, testosterone and magnesium salts are found to have pronounced anti-vasopressin activity to one or more of its actions. In the search for additional therapeutic agents for the treatment of dysmenorrhea, the above data indicate that the anti-vasopressin activity of compounds should be determined along with the anti-histaminic and the anti-cholinergic activity.

THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Thirty-fourth Annual Meeting

DETROIT, MICHIGAN, April 18–22, 1949

(For possible corrections in any of the following abstracts see the June issue)

Blood pressure measurements and changes in peripheral vascular bed of unanesthetized mice. GLENN H. ALGIRE. *National Cancer Inst., Bethesda, Md.* The transparent chamber technique as adapted to a skin flap in mice makes accessible for microscopic observation a layer of subcutaneous and muscular tissue approximately 0.5 mm. thick, and having a surface area of 150 sq. mm. Microscopic observations, photographs and quantitative measurements may be made at magnifications up to 500×. An indirect method has been devised for blood pressure measurements of any vascular component within the chamber during direct microscopic examination by transmitted light. Measurements may be made on the same vessel intermittently throughout the day, and repeatedly for the duration of the preparation (approx. 30 days). The apparatus consists of a mercury sphygmomanometer system and air reservoir connected to a glass tube having a thin rubber membrane across the end. A micromanipulator is used to bring the membrane into contact with the under surface of the skin. Pressure applied using the sphygmomanometer bulb results in slight bulging of the membrane. As the entire field is visualized under the microscope one can obtain arterial systolic and diastolic pressure, and venous pressure. The pulse wave can be seen at maximum amplitude approximately midway between systolic and diastolic pressures. Parallel observations and measurements may be made of correlated vascular phenomena such as changes in caliber of vessels, rates of flow, vasomotion, and intravascular agglutination.

Radiation effects on tissues studied with C^{14}-labeled glycine. KURT I. ALTMAN, GEORGE W. CASARETT, THOMAS R. NOONAN and KURT SALOMON (introduced by F. S. ROBSCHEIT-ROBBINS). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* Young adult male rats were exposed to 300 or 600 R total-body Roentgen radiation. Glycine labeled with C^{14} in the α-carbon atom (specific activity of 1.83 μc/mg.) was administered by stomach tube or intravenously at varying intervals after irradiation. The most striking feature observed in rats given 2 μc by stomach tube immediately after irradiation and killed 24 hours later was the high C^{14}-activity of the gastrointestinal tract contents. With a dose of 300 R 16.7% and with 600 R 32.8% of the total activity administered were found in the gastrointestinal tract contents, as compared with 0.82% in the control animal. In order to eliminate possible malabsorption, glycine (1 μc.) was injected intravenously and rats were sacrificed 5 hours later. It was found that the uptake of labeled glycine by muscle was definitely lowered in all irradiated rats, particularly when glycine was given immediately after irradiation. Definite changes were also observed in brain when glycine was injected 48 hours after irradiation. In this case the total C^{14}-activity of the brain was approximately 3 times that of the control. This increase was due to an increase in the C^{14}-activity of the phospholipid fraction. Radiation effects are also demonstrable in other organs, such as testes, kidney, and liver. When glycine was injected 48 hours after exposure to X-rays a striking increase in the C^{14} activity of the expired CO_2 was observed.

Ovarian hormones and uterine pigmentation in vitamin E-deficiency. W. B. ATKINSON (by invitation), H. KAUNITZ and C. A. SLANETZ (by invitation). *Depts. of Anatomy, Pathology and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City.* One of the most constant findings in intact vitamin E-deficient rats is a chocolate brown discoloration of the uterus which is characterized histologically by the accumulation of acid-fast pigment granules in the cells of the myometrium and, to a lesser extent, the endometrial stroma. The role of the ovary in the development of this condition has not been elucidated. In the present experiments, rats were ovariectomized at 21 days of age and were maintained on a vitamin E-deficient diet which contained 10% commercial lard and which limited the tocopherol intake to about 30 micrograms daily. The animals were divided into 4 groups; the first receiving no further treatment, the remaining 3 being injected once weekly with 5 micrograms of estradiol (Roche-Organon), 4 mg. of progesterone (Ciba) or both hormones together, respectively. The uteri were examined macroscopically and histologically after 5 to 11 months. Pigmentation was almost completely absent in the untreated ovariectomized animals. The animals receiving estrogen alone showed slight uterine pigmentation

in the areas characteristic of the unspayed deficient rat. Concurrent treatment with progesterone seemed to intensify the response. Treatment with progesterone alone evoked slight pigment deposition limited almost entirely to the circular layer of the myometrium. These observations clearly demonstrate that the ovarian hormones are involved in the accumulation of pigment in the uterus of the vitamin E-deficient rat.

Electron microscope studies on virus-cell relationships. F. B. BANG and G. O. GEY. *Depts. of Medicine and Surgery, Johns Hopkins Medical School, Baltimore, Md.* Newcastle and mumps viruses freshly prepared from allantoic fluid and saline show characteristic morphologies in the electron microscope. The shape of Newcastle virus when fixed by osmic acid is determined by the concentration of saline in which the virus is fixed. The absorption of both of these viruses on the ghosts of chicken red cells may be demonstrated by fixation with osmic acid in solution at intervals after exposure of the red cell ghosts to the virus, and drying the mixture on formvar screens. The effect of Newcastle virus on chick fibroblasts and macrophages and on rat fibroblasts has been studied by infecting the cells either in roller tube cultures before transfer to formvar coated coverslip preparations or at the time of transfer. A similar morphological effect has been demonstrated on all 3 cell types.

Cytochrome oxidase, succinoxidase and phosphatase activities of tissue of rats on protein-deficient diets. E. P. BENDITT, C. H. STEFFEE (by invitation), T. HILL (by invitation) and T. L. JOHNSTON (by invitation). *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* Protein-deficient but otherwise adequate diets when fed to animals result in loss of protein from many vital organs including the liver and kidneys. In an effort to elucidate the nature of this protein loss rats were sacrificed after varying periods on protein deficient diets. Cytochrome oxidase, succinoxidase and alkaline phosphatase were assayed in liver homogenates and alkaline phosphatase determined in kidney homogenates. Cytochrome oxidase and succinoxidase decreased progressively with time and somewhat faster than the liver protein. Liver phosphatase, on the other hand, increased in concentration as the liver shrank. The total liver phosphatase remained approximately constant. Kidney phosphatase after several months of protein depletion was reduced in proportion to the kidney protein. The difference in behavior of the liver phosphatase from the kidney enzyme and from the other liver enzymes studied is apparently related to the fact that the liver enzyme is not present to a significant extent in the hepatic parenchymal cells but is associated with the vascular bed and bile ducts. This work substantiates that of others who assayed under similar conditions other enzymes including catalase, xanthine dehydrogenase, cathepsin and arginase (Miller, *Fed. Proc.* 7: 174, 1948) d-amino acid oxidase (Seifter et al. *Fed. Proc.* 7: 187, 1948). It appears from the evidence that at least a part of the 'reserve' protein lost from vital organs such as the liver and kidney by animals on protein deficient diets is not inert material but is part of the actively functioning cytoplasmic protein constituents, i.e. enzymes.

Effect of acute x-radiation on distribution and excretion of radio sodium in the rat. L. R. BENNETT, VERA C. BENNETT and JOE W. HOWLAND (introduced by F. S. Robscheit-ROBBINS). *Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Radiated rats have been shown to have an increased thiocyanate space for 2 weeks following a mid-lethal dose of x-radiation. A study of the sodium distribution and excretion of similar animals using radio sodium has been made. In rats during the first 4 days post radiation a marked retention of radio sodium occurs. Tissue distribution studies show a shift into the intestinal tract which may exceed that observed in control animals by as much as 2% of the total dose administered. Smaller shifts of radio sodium into the kidney and spleen are noted. There is an increased radio sodium content in the liver which appears to be definitely related to the increased serum level to which it holds a constant relationship. The serum sodium content increases, the maximum levels (up to 5–8 mEq.) being observed at the end of 3–4 days after irradiation. Urinary radio sodium excretion may be reduced to one half that observed in control animals. Studies on dehydrated rats indicate that the urinary retention and elevated blood sodium level are not accounted for on the basis of dehydration alone.

Lipids in the lymph of rats. J. L. BOLLMAN and E. V. FLOCK. *Mayo Foundation, Rochester, Minn.* Lymph from the lymphatic draining the small intestine and lymph from the liver and lymph from the thoracic duct was obtained in different rats after recovery from the operation during which a small plastic tube was inserted into the respective lymphatic and brought through the abdominal wall. The concentration of neutral fat of the intestinal (and thoracic duct) lymph of the fasting rat was considerably greater than that of the plasma. The phospholipid but not the cholesterol content of this lymph was also higher than that of plasma. The feeding of a lipoid free meal did not alter these values. Following the feeding of a meal containing fat but devoid of phospholipid or cholesterol there was a marked increase in the neutral fat and phospholipid but not the cholesterol content of the intestinal lymph. The feeding of phospholipid produced only the increase in the phos-

pholipid content of the lymph which would accompany the increased neutral fat (from hydrolyzed phospholipid) content of the lymph. The concentration of neutral fat, phospholipid and cholesterol in lymph from the liver of fasting rats was almost the same as the concentrations of those substances in the plasma and were not materially altered by feeding meals which did elevate the plasma content of these substances.

Blood supply of neoplasms in the liver. CHARLES BREEDIS and GANG YOUNG (by invitation). *Dept. of Pathology, Medical School, Univ. of Pennsylvania, Philadelphia, Penna., and Pathology Research Inst., Medical College, National Sun Yat Sen Univ., Canton, China.* By means of injection experiments during life and after death, it was shown that malignant neoplasms growing in the liver tend to acquire an exclusively arterial blood supply. The neoplasms tested were as follows: V-2 carcinoma strain of rabbits, T-241 sarcoma strain of mice, metastases of spontaneous carcinoma of the kidney of the frog, and primary hepatomas and cholangiomas of rats induced by butter yellow. A variety of metastatic carcinomata found at autopsy in 12 human cases were similarly injected. In all instances the blood supply to the tumors was predominantly or exclusively arterial, in sharp contrast to the predominantly portal supply of liver tissue. It was found that the blood supply of the experimental rabbit tumors was arterial whether inoculations had been made into the portal vein, the hepatic artery or directly into the liver parenchyma. Only very small tumors (1–2 mm. or less) tended to retain some portal vessels. With increase in size of the tumors the portal vessels became obliterated, and blood was supplied through the hepatic artery.

Cutaneous melanin production in the mouse by 5, 9, 10-trimethyl, 1-2-benzanthracene. F. H. BURGOYNE, W. E. HESTON, J. L. HARTWELL (all by invitation) and H. L. STEWART. *National Cancer Inst., Bethesda, Md.* By painting the skin at the base of the tail of F_1 hybrids of strains $C_{57}B1$ x dba twice weekly with 0.06% solution of 5,9,10-Trimethyl, 1–2 Benzanthracene in benzene, localized pigmented areas were produced. The pigmented areas appeared in the skin between the 5th and 15th months, often in association with areas of atrophy, hyperplasia, inflammation, ulceration, papilloma formation, squamous cell carcinoma and sarcoma. In histological preparations the pigment in these areas was blackened by Krajian's silver impregnation method, failed to give a positive Prussian Blue reaction and was bleached by strong oxidizing agents, thus identifying it as melanin. When the pigmented areas were bleached they were found to be composed of melanophores or macrophages with finely stippled cytoplasm and an eccentrically placed nucleus. The 'dopa' reaction applied to skin recently excised from the painted area revealed large numbers of melanoblasts in the basal layer of the epidermis. Thus, painting the skin of these F_1 hybrid mice with 5,9,10-Trimethyl, 1–2 Benzanthracene resulted in an increase of melanoblasts, with deposition of melanin in macrophages in the derma and the subcutaneous tissue. No malignant melanomas were induced.

Effects of x-rays on the metabolism of nucleic acids in hematopoietic tissues. C. E. CARTER (introduced by SHIELDS WARREN). *Biology Division, Oak Ridge National Laby., Oak Ridge, Tenn.* The alterations in the metabolism and content of nucleic acids in hematopoietic tissues have been studied through microchemical and isotope procedures employing P^{32} following varying doses of total body x-radiation. In bone marrow the desoxyribonucleic acid content falls within the first 24 hours following radiation. Similar changes were found in spleen and thymus where the desoxyribonucleic acid changes are marked but a decrease in ribonucleic acid content is also found. No immediate effects of radiation on the content of nucleic acid in these tissues were noted. Experiments employing P^{32} in bone marrow cell suspensions in homologous serum likewise showed no significant alteration of the rate of incorporation of P^{32} into nucleic acids following removal from rabbits immediately after exposure to 1000 R. Experiments conducted 24 hours following exposure show marked decrease of respiration in 500 R and 1000 R group with elevated ratios of ribonucleic acid to desoxyribonucleic acid specific activity indicating a depression of desoxyribonucleic acid synthesis. Of several enzymes concerned with nucleic acid and protein metabolism studied in spleens of irradiated rats, only ribonuclease shows significant deviation from normal and these changes are established in the first 24 hours following exposure. All of the aforementioned changes pose the problem of correlation of metabolic activity with cell type in a complex cellular system.

Experimental production of a nutritional macrocytic anemia in swine. G. E. CARTWRIGHT (by invitation) and M. M. WINTROBE. *Dept. of Medicine, Univ. of Utah College of Medicine, Salt Lake City, Utah.* A deficiency of pteroylglutamic acid has been produced in 32 swine fed a basal diet supplemented with sulfasuxidine, a folic acid antagonist (crude methyl folic acid) and all the known B vitamins except pteroylglutamic acid. The hematologic manifestations observed were: *1*) a severe macrocytic anemia; *2*) leukopenia, due to a reduction in both polymorphonuclear and mononuclear cells; and *3*) a slight thrombocytopenia. The bone marrows were hyperplastic with a relative increase in immature nucleated red cells. These cells closely resemble the megaloblasts seen

in the bone marrow of patients with pernicious anemia in relapse. The hematologic manifestations described above, appeared in the presence of 'extrinsic factor' in the diet and their development was not prevented by the daily intramuscular administration of 1 U.S.P. U of purified liver extract from the beginning of the experiment. The feeding of a diet low in protein (10% casein) resulted in a more rapidly developing anemia but the degree of macrocytosis was less. Pteroylglutamic acid deficient pigs, either receiving 'extrinsic factor' (crude casein) in the diet or an 'extrinsic factor-free' diet (purified casein) responded rapidly and maximally to pteroylglutamic acid, pteroyldiglutamic acid, pteroyltriglutamic acid or to pteroylheptaglutamic acid. Thymine and xanthopterin had little or no activity. Tyrosine, adenine and uracil were inactive. Purified liver extracts as well as vitamin B_{12} were found to possess some hemopoietic activity under the conditions employed but the activity was considerably less than that of pteroylglutamic acid compounds.

Biochemical and clinical effects of cationic exchange resin. THEODORE S. COBBEY, JR. (by invitation), ROBERT H. WILLIAMS, NORMA MACRAE (by invitation) AND BEVERLY T. TOWERY (by invitation). *Dept. of Medicine, Univ. of Washington, Seattle, Wash.* Several cationic exchange resins have been given to normal subjects and patients with edema. Investigations were made for changes in the clinical status and in the balance of H_2O, Na, K, Ca and Mg. The resins markedly increased the excretion in the stools of Na, K, Ca and Mg. Although there tended to be a decrease in the excretion of these elements in the urine, the response was variable. The increased excretion of the elements in the stools was not associated with a comparable decrease in the urine. The resins promoted a marked increase in ammonia and titratable acidity in the urine. In certain patients with edema associated with congestive heart failure it was noted that loss of weight and edema occurred simultaneously with a positive Na balance; conversely, gain in weight and edema occasionally was disproportionate to the quantity of Na retained. These observations suggest that the Na balance need not necessarily parallel the water balance in congestive heart failure. After a period of 3 days on the resin one non-cardiac subject had a marked diuresis followed by weakness, hemoconcentration, and a distaste for water. These symptoms were immediately relieved by intravenous saline. One cardiac patient developed the same picture after being on the resin for 21 days.

Determination of the blood iodine. A simplified method for use in the clinical laboratory. ARTHUR C. CONNOR (by invitation), ROY E. SWENSON (by invitation) and GEORGE M. CURTIS. *Dept. of Research Surgery, Ohio State Univ., Columbus, Ohio.* A shorter and simpler plan of determining the blood iodine has been attained by developing a method which requires no transfer of material during the entire procedure, and necessitates an apparatus of specially constructed glassware. Three ml. of serum are fractionated in a 250 ml. reagent flask by use of the Somogyi reagent. This flask is centrifuged in an International, size 2 centrifuge. The supernatant fluid containing the soluble 'inorganic' iodine is poured off and read in the colorimeter directly. The protein precipitate containing the physiologically significant protein-bound iodine remains in the 250 ml. reagent flask and is oxidized by the chromic acid procedure. The same flask fits a distillation assembly. The iodine is then released by phosphorous acid and collected directly in a colorimeter tube which also fits the distillation assembly. The quantity of iodine is determined by its catalytic effect on the rate of reaction between ceric sulfate and arsenious acid. An ordinarily well-trained technician can make an analysis in less than 3 hours and about ten analyses in an eight-hour day. The average deviation is plus or minus 5%. Since the method is essentially a closed procedure contamination has been practically eliminated.

Kidney protein associated with systemic arteriolar necrosis. G. I. DESUTO-NAGY and L. L. WATERS (introduced by M. C. WINTERNITZ). *Dept. of Pathology, Yale Univ. School of Medicine, New Haven, Conn.* Previous experiments have demonstrated that when saline extracts of kidney tissue are injected into nephrectomized dogs, segmental necrotizing lesions of systemic arterioles result (Winternitz, *et al.*, 1939). In continuance, acid extracts of fresh hog kidney were subjected to fractional acetonic precipitation. The precipitates were redissolved, dialyzed, and reprecipitated with acetone. Three fractions resulting from these procedures have been examined for their vasomotor activity and for their association with the occurrence of acute arteriolar lesions. The dialysates of all the fractions contained vasodepressor substances of small molecular weight. When these were injected into nephrectomized test dogs no systemic vascular changes were encountered. A complex fraction, containing vasopressor (renin-like) substances, thromboplastic material, inert globulins and lipids was found to be associated with the frequent occurrence of vascular changes in the test animals. A small non-dialyzable fraction of the acid extract obtained on precipitation by 20 volumes of acetone was devoid of either vasopressor or vasodepressor activity. This protein-like material contained phosphorus. When injected into nephrectomized dogs it was found to be associated with widespread arteriolar necrosis. In some instances necrosis of arterial smooth muscle followed the injection of as little as 19.1 mg. N.

Iron excretion in human subjects as measured by the isotope technique. REUBENIA DUBACH (by invitation), CARL V. MOORE and SHEILA CALLENDER (by invitation). *Washington Univ. School of Medicine, St. Louis, Mo.* The excretion of iron by human subjects over a 140-day period was studied by the isotope technique. Observations were continued for a period of time greater than the length of life of the red cells into which labeled hemoglobin was incorporated in order that any unusual excretion of radioiron at the time of hemoglobin breakdown might be noted. Following intravenous injections of 5 to 20 mg. of radioiron, 4 normal individuals excreted in the feces 0.03 to 0.07% of the dose per day for 4 to 24 days; later, after the isotope had reached a constant level in the blood (75 to 92%), an average of approximately 0.01% of the dose was excreted daily for 140 days. A woman with hypochromic anemia excreted an average of 0.028% of the dose daily for the first 5 days; later, when 100% of the isotope had appeared as hemoglobin, she excreted 0.002 to 0.007% of the dose daily for 140 days. A patient with sickle cell anemia excreted an average of 0.05 to 0.08% of the injected dose per day. Assuming complete mixing of isotopic iron with total hemoglobin iron, the total fecal excretion of iron derived from hemoglobin was calculated to be 0.2 to 0.9 mg/day for the normal subjects, 0.04 to 0.13 mg. for the woman with hypochromic anemia, and 1.3 to 1.7 mg. for the girl with sickle cell anemia. The effect of a hemolytic crisis on iron excretion was studied in 2 dogs. After a high concentration of radioiron had been built up in the red cells by repeated injections, severe hemolysis was induced with phenylhydrazine. The excretion of iron in the feces and urine increased significantly (0.1 to 0.12% of the dose per day, compared to 0.01 to 0.06% in control periods) but remained small in relation to the amounts of iron liberated by red cell destruction.

Effect of hydrogen-ion concentration on the cultivation of macrophages in vitro. I. N. DUBIN and C. K. YEN (by invitation). *Division of Pathology and Bacteriology, Univ. of Tennessee College of Medicine, Memphis, Tenn.* Macrophages from chick embryo spleen were grown in vitro at 37°C. for 4 to 6 days using chicken serum and salt mixtures containing bicarbonate buffers as nutrient. The *p*H values, which ranged from 6.4 to 9.0, were maintained at constant levels by continuous flow of gas-mixtures containing required concentrations of CO_2. The *p*H values were determined with a Beckman pH meter. The macrophages grew equally well between *p*H 6.8 to 8.4. Growth fell off sharply at *p*H 6.6 and ceased about *p*H 6.4; beyond *p*H 8.4 growth was poor, but cells in rare numbers were found up to *p*H 9.0. There was indirect evidence that the internal *p*H of the cells was affected, as shown by the color reactions of the cells when stained with Giemsa; on the acid side the cells stained reddish while on the alkaline side bluish. On the acid side the cells were round, large, contained abundant cytoplasm and numerous fat vacuoles; there was also a tendency towards formation of multinucleated giant cells. On the alkaline side the cells had much less cytoplasm and fewer fat vacuoles and presented a stellate or elongated shape. This difference in shape may reflect a difference in irritability and mobility. The mechanism of action of the *p*H is not known but may operate through changing the concentration of available ions such as calcium and phosphate.

Acid phosphatase activity of the gastric contents of patients with carcinoma of the stomach. CHARLES E. DUNLAP and GEORGE W. CHANGUS (by invitation). *Dept. of Pathology, School of Medicine, Tulane Univ. and Charity Hospital, New Orleans, La.* In patients with carcinoma of the stomach histochemical studies have shown high acid phosphatase activity in the neoplasm and also in the surrounding gastric mucosa. Chemical determinations for acid phosphatase were done by the method of Gutman and Gutman on gastric contents, aspirated from a series of fasting patients with and without carcinoma of the stomach. It was found that aspirates having an initial *p*H of less than 3.5 seldom contained significant amounts of acid phosphatase and that the enzyme, when present in other samples, could be irreversibly inactivated, in vitro, by acidification to *p*H 3.5 or less. At a *p*H greater than 3.5 the enzyme was fairly well preserved for 48 hours at 4°C. but rapid deterioration occurred at room temperature. Thus gastric aspirates containing 'free acid' (*p*H less than 3.5) as well as those that had stood for more than 2 hours without refrigeration were considered unsuitable for acid phosphatase determinations. In a great majority of the patients with carcinoma of the stomach no 'free acid' was present and the aspirates were found to contain more than 10 units of acid phosphatase per hundred cc. Most samples from patients without gastric carcinoma contained less than 10 units. The study, to date, has covered only a limited number and variety of gastric lesions and includes no cases of early carcinoma.

Blood dyscrasia in mice ingesting toluidine blue. THELMA B. DUNN. *National Cancer Institute, Bethesda, Md.* Inbred mice were given 0.1% or 0.2% solution of toluidine blue instead of drinking water. With an appropriate concentration of the dye the urine was deeply colored, but no severe toxic symptoms developed. After an interval of 10 days or longer the peripheral blood in some animals showed a reduction in the circulating red blood cells without a corresponding reduction in hemoglobin. In the blood smears the erythrocytes showed fragmentation, polychromasia, anisocyto-

sis, macrocytosis, siderocytosis and normoblasts. The total and differential leukocyte counts were not significantly altered. On postmortem examination a pronounced siderosis of the spleen, liver, bone marrow, kidney and lymph nodes was found. The bone marrow showed hyperplasia of erythropoietic elements. When the toluidine blue was withdrawn, the peripheral blood picture rapidly returned to normal, and the siderosis of organs decreased. This method is now being used to produce siderosis in the tissues and hyperplasia of the bone marrow in mice. The possible effect on the incidence of neoplastic disease, particularly leukemia, is being investigated.

Incidence of neoplasms and pathological changes in rats as a result of prolonged choline deficiency. CYRUS C. ERICKSON and WALTER GOEBBEL (by invitation). *Depts. of Pathology and Surgery, Duke Medical School, Durham, N. C.* Neoplasms of one or more types were observed in 58% of choline-deficient rats by Copeland and Salmon (D. H. Copeland and W. P. Salmon, *Am. J. Path.* 22: 1059, 1946). The purpose of these experiments was to test a similar choline-deficient diet for the production of neoplasms in the Osborne-Mendel strain of rats used in this laboratory, and to utilize such test animals for anterior chamber tumor transplant experiments. The diet consisted of 30% alcohol-extracted peanut meal, 6% alcohol-extracted casein, 40% sucrose, 20% pure lard, and 4% salt mixture. To each kg. of diet, vitamins were added as in the Copeland-Salmon choline-deficient diet. The experimental diet was started on 149 rats at the age of 20–30 days and was given ad libitum. Choline supplement was administered to the choline-deficient group during the first 4 weeks. Twenty-four rats making up the control group received the same diet with 3 gms. of choline chloride added to each kg. of diet for the duration of the experiment. Sixty of the experimental group and 20 of the control animals were still living after 19 months. Cirrhosis of the liver was a consistent finding in the rats on the deficient diet. The high incidence of neoplasms previously reported as a result of this choline-deficient diet has not been observed in this experiment in the 65 choline-deficient animals expiring prior to 19 months.

Injury and recovery of mouse testes following whole-body exposure to x-radiation. ALLEN ESCHENBRENNER, *National Cancer Institute, Bethesda, Md.* Groups of mice received 50 R, 100 R, 200 R, 300 R, or 400 R acute whole-body radiation at 3 months of age. Four mice of each dose group and four non-irradiated mice of the same age were killed at weekly intervals up to and including 12 weeks following exposure. The testes were dissected free and weighed on a torsion balance after which they were prepared for histologic examination. Minimum testes weights were observed from 3–4 weeks after irradiation and returned to normal 6 to 14 weeks after irradiation. The minimum weights of testes observed were dependent upon dose. The time required to effect the minimum weights as well as the time required for the weights to return to normal were functions of dose. Histologic examination of these testes indicates that the decrease of testes weights following acute irradiation is due to marked transient retardation in rate of production of spermatogonia. The progeny of spermatogonia are not appreciably destroyed nor prevented from maturation. After it was found that the weight of testes of mice exposed to 200 R had returned to normal by 12 weeks, an additional group of mice was given a whole-body exposure of 200 R at 3 months of age and again 12 weeks later. Following the second exposure the mice were killed and examined as before. The pattern of injury and recovery following the second dose, as reflected in fresh testes weights, was the same as observed following a single dose. These experiments on acute irradiation confirm conclusions drawn from earlier experiments on chronic irradiation; that the primary effect of radiation of the testes is on the spermatogonia.

Massive hepatic necrosis in protein-depleted, partially hepatectomized rats fed hypertonic diets. ROBERT L. ESTRADA (by invitation), ZACHARY A. SIMPSON (by invitation), HARRY M. VARS and I. S. RAVDIN. *Harrison Department of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* The effect of forced feeding with concentrated solutions of sucrose, gelatin and casein hydrolysate on normal partially hepatectomized and on protein-depleted, laparotomized or partially hepatectomized rats has been studied. Massive hepatic necrosis occurred in the rats receiving these diets. Gastric distention and increased intra-abdominal pressure were observed in the experimental groups and were simulated in others by attaching a balloon to the stomach at laparotomy. Pneumoperitoneum immediately after laparotomy demonstrated that increased intra-abdominal pressure per se was not sufficient to produce liver injury. The emptying time of the stomach following the administration of the various diets was determined by x-ray examination. No retention was present after 2 hours in the control group. In the groups receiving 50% sucrose and the 50% hydrolyzed casein diet, there was 50% and 90% gastric retention, respectively, at the end of 2 hours. The debilitated rat is very susceptible to liver injury. It is our opinion that the hepatic necrosis which occurred in rats receiving the 50% casein hydrolysate diet was not due to toxicity of the amino acids, since similar lesions were produced when other diets were fed, and that gastric distention per se was sufficient to slow the portal circulation and to lead to anoxia of the hepatic

parenchymal cells. The condition was further aggravated by pressure exerted directly upon the liver. This represents another mechanism which by interfering with hepatic blood supply leads to hepatic necrosis, the extent of the necrosis being further aggravated by the nutritional state of the animals.

Effect of lecithin in the formation of thrombin. JOSEPH E. FLYNN and EUGENE T. STANDLEY, (introduced by H. P. SMITH). *Dept. of Pathology, Columbia Univ., New York City.* In activating purified prothrombin, the amount of thrombin obtained depends in part upon the amount of Factor V added. With a suboptimal amount of Factor V, the yield of thrombin is low, and can be increased by the addition of lecithin. The lecithin is not identical with Factor V, but it does potentiate its effect.

Inorganic composition and phosphatase activity of human cartilage in relation to morphological differentiation. RICHARD H. FOLLIS, JR. *Dept. of Pathology, Johns Hopkins University, Baltimore, Md.* A method has been devised to compare the chemical composition and morphological characteristics of developing human cartilage. Segments of whole costochondral junctions removed at autopsy from children of varying ages are clamped in a freezing-type microtome by the shaft and first sliced longitudinally down through the cartilage just into the shaft. Transverse slices of desired thickness then are made completely through one of the two longitudinal halves and part-way through the other. Appropriate chemical analyses are made of the free slices; their fellow halves, held together by an intact outer layer of cartilage, are fixed and prepared for histological examination. This technique permits a comparison of chemical composition and cellular differentiation in various strata of developing cartilage; a unique tissue with such adaptability to studies of chemical embryology. Observations to be presented include the percentage composition of calcium, phosphorus and carbonate and phosphatase activity, all in direct relation to the degree of maturation of cartilage cell strata, from those containing small undifferentiated cells to those having large hypertrophic cells being invaded by capillaries. There is a characteristic quantitative increase in concentration of the inorganic elements which proceeds slowly and then suddenly mounts rapidly as the zone of hypertrophic cells is reached. Similar, though more marked differences, are found in phosphatase activity.

Nucleic acids and the production of antibody by plasma cells. CAROLYN FORMAN (by invitation), WILLIAM E. EHRICH and DAVID L. DRABKIN. *Depts. of Pathology and Biochemistry, Graduate School of Medicine, Univ. of Pennsylvania, and the Philadelphia General Hospital, Philadelphia, Pa.* It has been suggested that protein synthesis is associated with nucleic acid metabolism. Desoxyribose nucleic acid (DNA) appears to be connected with the multiplication of chromosomes, whereas the production of cytoplasmic protein is thought to be conditioned by ribose nucleic acid (PNA). We have investigated the relationship of antibody formation and the production of nucleic acids in lymph nodes of rabbits, injected with typhoid vaccine. The increase in DNA was found to be parallel with the increase in weight of the nodes suggesting that the latter was due largely to multiplication of cells. PNA, on the other hand, showed its greatest rise between the 4th and 6th day after vaccine injection when antibody formation is at the peak. Thereafter, PNA rapidly decreased. A histologic study of methyl green and pyronin stained sections of the nodes revealed that during the first 6 days of the experiment the cellular reaction was chiefly that of plasma cell proliferation, and that the plasma cells contained most of the PNA. The lymphocytes, on the other hand, showed their greatest activity only after PNA and antibody formation had passed their peaks. These results are interpreted as indicating that the plasma cell and not the lymphocyte is responsible for antibody formation.

Minimum daily requirements of indispensible amino acids for maintenance of adult rats. L. E. FRAZIER, R. L. WOOLRIDGE, C. H. STEFFEE, (all by invitation) and E. P. BENDITT. *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* The quantitative requirements for the 9 amino acids indispensible for maintenance of appetite, weight and nitrogen equilibrium of adult, well-nourished, male, albino rats (Wissler *et al. J. Nutrition* 36: 245, 1948) were measured. Mixtures of crystalline amino acids were used as the nitrogen source. The caloric intake was maintained at or slightly above 1200 Cal./m²/day and the nitrogen intake at or slightly above 5 gm/m²/day. Vitamin, mineral and fat intakes were adequate. The level of the amino acid under consideration was varied while other diet factors were kept constant. Weight changes and nitrogen balance were determined. Seven to 14 animals were used for each amino acid. The minimum quantity of amino acid necessary to maintain weight and nitrogen equilibrium was estimated from the curves relating amino acid intake and weight change or nitrogen equilibrium respectively. In all instances the quantity of amino acid necessary to maintain nitrogen equilibrium appeared to be somewhat lower than the amount necessary to maintain the weight. The requirements for each amino acid for nitrogen equilibrium and weight balance respectively were as follows: Tryptophane 1.8 and 2.2, histidine 2.1 and 2.2, phenylalanine 3.1 and 6.0, lysine 3.7 and 4.5, threonine 5.1 and 5.3, methionine 5.3 and 7.3, leucine

6.5 and 8.0, valine 7.0 and 10.0, isoleucine 10.8 and 13.7 mgm/100 cm² of body area/day. Furthermore, animals offered an amino acid mixture containing all the indispensible amino acids at the above levels were maintained in nitrogen equilibrium.

Fates of parenterally administered homologous serum protein and casein hydrolysate. JOHN FULLER (by invitation), E. M. HUMPHREYS (by invitation), C. H. STEFFEE (by invitation), R. W. WISSLER (by invitation), and E. P. BENDITT. *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* Groups of protein-depleted rats were fed a protein-free basal ration by gavage. One group was given homologous serum intravenously, a second, casein hydrolysate (Amigen) intravenously, and a third, Amigen orally. Each animal received 5 gms. protein/kgm. body wt/day for 9 days. Nitrogen balance, blood compartments, and extracellular fluid volume determinations were made. Carcasses, and livers were analyzed for protein, fat, water and ash. Percentages of nitrogen intake retained in 9 days were:

Compartment	I.V. serum	I.V. hydrolysate	Oral hydrolysate
Plasma	6.9	2.6	1.1
Liver	8.9	4.9	4.6
Carcass	32.8	10.7	36.8
Total	48.6	18.2	42.8

Animals receiving oral hydrolysate had the smallest extra- and intra-cellular fluid and plasma volume increases. The largest plasma volume as well as plasma protein increases were associated with intravenous administration of serum. Although the animals receiving intravenous hydrolysate gained far less plasma protein than those receiving serum, their plasma volume increases were nearly as great. No increased nitrogen excretion was found in the serum-injected animals in the ten days following cessation of injection during which they received an adequate protein diet. It appears that these rats utilized injected serum protein for tissue synthesis. The rats appeared to utilize the serum protein more than twice as effectively as the injected casein hydrolysate. The poorer utilization of the injected hydrolysate may have been due to its content of polypeptides.

Prophylaxis of allergic acute disseminated encephalomyelitis in guinea pigs with salicylate and para-aminobenzoic acid. ROBERT A. GOOD, BERRY CAMPBELL and T. A. GOOD (introduced by IRVINE MCQUARRIE). *Univ. of Minnesota, Minneapolis, Minn.* That acute diffuse central nervous system disease can be produced in experimental animals by injection of heterologous and homologous brain tissues was shown by Rivers, Sprunt, and Berry. Schwentker and Rivers, among others, demonstrated that this phenomenon was associated with formation of organ-specific antibodies against brain tissue. Recent studies have resulted in the production of auto-allergic disease of the central nervous system of monkeys, rabbits, and guinea pigs by a single or few injections of homologous brain tissue with adjuvants. Recent studies have shown that 70 to 90% of guinea pigs develop symptoms of progressive encephalomyelitis 13 to 37 days after injection of homologous brain plus an adjuvant of heat killed acid fast organisms, Falba, and Bayol F. In an attempt to test the effect of para-aminobenzoic acid and sodium salicylate medication on the course of this disease, 124 guinea pigs were studied. Ninety % of control animals developed progressive encephalomyelitis between 13 and 20 days after a single injection of guinea pig brain with adjuvants. While neither subcutaneous administration of sodium salicylate nor oral administration of para-aminobenzoic acid in moderate dosages alone affected the course of the allergic encephalomyelitis, premedication and medication with a combination of salicylates and para-aminobenzoic acid reduced its incidence to 10%. Administration of sodium salicylate in larger dosages resulted in demonstrable but less effective protection in spite of salicylate toxicity. Neither agent alone nor combination of the two had any ameliorating effect when administered after the encephalomyelitis had developed.

Canine hemophilia: the clotting anomaly and effectiveness of transfusions. JOHN B. GRAHAM (by invitation), JOSEPH A. BUCKWALTER (by invitation), L. J. HARTLEY (by invitation) and K. M. BRINKHOUS. *Dept. of Pathology, Univ. of North Carolina, Chapel Hill, N. C.* A sex-linked hemorrhagic disease of male dogs was described by Field et al. These animals have been studied in our laboratory for the past two years. The clotting defect appears to be identical with human hemophilia. Bleeding time from small puncture wounds is normal while bleeding from larger wounds is extensive. The clotting time is prolonged. Prothrombin is present in normal amounts, but its conversion to thrombin is extremely slow. The rate of prothrombin utilization can be increased by the addition *in vitro* of thromboplastin, normal dog plasma, or Fraction I (Armour; Squibb). Prothrombin conversion in whole hemophilic blood approximates the normal rate after addition of normal plasma. This corrective action appears to be dependent upon the presence of formed elements, particularly platelets. Mixtures of platelet-poor hemophilic plasma and platelet-poor normal plasma do not clot for hours, and almost no prothrombin is converted to thrombin. Normal blood and plasma transfusions correct the clotting defect. The corrective effect is most pronounced for approximately 24 hours, but a slight effect persists as long as 72–120 hours. Normal serum trans-

fusions have no effect. Transfusions of fresh and aged hemophilic blood do not alter the clotting anomaly. Untreated bleeder dogs usually die by the age of 3 months. By the use of repeated plasma transfusions, the hemophilic dogs have been reared to maturity.

Spontaneous adenomatous gastritis of mice of strain dba. WILLIAM V. HARE (by invitation) and HAROLD L. STEWART. *National Cancer Institute, Bethesda, Md.* Inbred mice of strain dba develop a spontaneous lesion of the glandular stomach and duodenum, which is progressive and may be fatal. Grossly it is characterized by a pebbled appearance of the gastric mucosa or by large hypertrophic rugae with deep furrows between them, or a combination of the two. Microscopically the epithelial cells are frequently hyperchromatic and the glands are atypical and may penetrate the submucosa. The submucosa usually shows an inflammatory reaction which may also be present in the forestomach as well. In the duodenum single or multiple papillary adenomas develop having a broad base, and tending to fill the lumen, although they do not invade the muscularis. No evidence of malignant degeneration in either the gastric or duodenal lesions has been found. In some cases, however, there is obstruction of the pyloric canal.

Effect of aging on the protein fractions of the normal human heart. JOHN W. HARMAN and JEAN H. WEBSTER (introduced by D. MURRAY ANGEVINE).* *Dept. of Pathology, Univ. of Wisconsin Medical School, Madison, Wis.* A series of 34 human hearts, of ages varying from birth to 80 years and normal as determined by gross and histological features, are analyzed for various protein fractions. Representative samples of the principal chambers are ground finely, acetone dried and defatted with Skelly Solvent #2. Aliquots dried to constant weight are analyzed for total protein, alkali soluble protein, collagen and elastin by Conway's micro-kjeldahl technique. At different ages the ventricles are unaltered in the proportions of these fractions. Up to the age of 10 the 4 chambers are chemically similar, whereas beyond this age in certain hearts the atria contain a significantly increased quantity of collagen. Differences between atria of the same organ are not observed; these chambers fluctuate concomitantly in their composition. In all chambers, with the usual conversion factor of 6.25, only about 75–80% of the standard dried preparation is composed of protein. Part of the remaining per cent is a fraction soluble in 30% KOH, precipitable with alcohol, which is Biuret negative and yields no reducing substances by acid hydrolysis. The constant proportion of the protein fractions in aging normal hearts, except for certain atria, indicates that senility does not quantitatively affect the organ. Collagenization of the atria may be of significance functionally, since these chambers so frequently suffer rhythmic disorders.

Nitrogen mustards as effective blood and plasma sterilizing agents. FRANK W. HARTMAN and GEORGE H. MANGUN (by invitation). *Dept. of Labys., Ford Hospital, Detroit, Mich.* The authors recently demonstrated the ability of methyl-bis(beta-chloroethyl) amine hydrochloride (HN2) to sterilize blood and plasma with relatively slight changes in their components with the exception of an increase in prothrombin time. Other mustards (tris(beta-chloroethyl)amine and bis(beta-chloroethyl) sulfide) are approximately equal in effectiveness. The virucidal and bactericidal action of HN2 is dependent to some degree upon the medium, the *p*H, and the presence of substances competing for the agent. In citrated plasma the sterilizing dosages of HN2 are *1*) New Jersey vesicular stomatitis, 300 mg/l; *2*) lymphocytic choriomeningitis, 400 mg/l; *3*) St. Louis encephalitis, 300 mg/l; *4*) *E. coli*, 450 mg/l; *5*) Hemolytic streptococcus, 450 mg/l; *6*) *Staphylococcus aureus*, 800 mg/l; *7*) *Bacillus subtilis*, 150 mg/l. In the absence of plasma HN2 is still more effective, illustrating the protective or competitive effect of the plasma. In saline, *E. coli*, for example, is completely killed at 100 mg/l. In ACD citrated whole blood, 450 mg/l are required for the complete destruction of N. J. vesicular stomatitis virus. Studies carried out on ACD citrated whole blood treated with 500 mg/l of HN2 show: *1*) a very slight increase in fragility to hypotonic saline; *2*) no immediate hemolysis nor increase above control hemolysis on prolonged storage; *3*) a normal rate of loss of potassium from the red blood cells.

Renal excretion of phosphate in the dog; influence of potassium, action of parathyroid extract. C. A. M. HOGBEN (by invitation) and J. L. BOLLMAN. *Mayo Foundation, Rochester, Minn.* The existence of a maximal tubular reabsorption of phosphate was confirmed utilizing plasma ultrafiltrates for the measurement of plasma inorganic phosphate. During the course of a 6–8 hour experiment, the maximum will decline 25–50%. Incorporation of potassium in the infusion fluid would, under certain conditions, restore the maximum. The action of intravenous parathyroid extract was measured on the tubular reabsorption of endogenous phosphate in the intact dog for 2 hours after administration. A slight rise in plasma phosphate and urinary excretion of phosphate resulted. No evidence was obtained to support the hypothesis that parathyroid extract inhibited tubular reabsorption of phosphate. Intravenous parathyroid extract to the intact dog was without effect upon the maximum tubular reabsorption of phosphate for 2 hours after administration. Further studies will be reported on the action of parathyroid extract in the thyroparathyroidectomized dog.

Pathology of an experimental flash-burn. LEWIS HOGG, (by invitation), J. THOMAS PAYNE (by invitation) and HERMAN E. PEARSE. *Univ. of Rochester Atomic Energy Commission Project, Rochester, N. Y.* The importance of the flash burn as a clinical entity was pointed out by the Navy at Pearl Harbor, and by observers of the Hiroshima and Nagasaki casualties. An attempt was made to produce a flash burn, to study its morphology and the factors altering its healing. A series of about 75 such burns were produced on the pig, using magnesium explosions as the high intensity, short duration heat energy source. These lesions typically presented a zone of central pallor surrounded by abrupt erythema. The erythema and edema resolved over 4–7 days, leaving only a thin central crust which came free at about 10 days. Histologically, the lesions presented an abrupt, linear demarcation at their margins, as well as their depths, as manifest by hair follicle and dermal changes. Healing was by sequestration of the burned tissue, rather than by organization, and required 3–5 days. This is a unique picture and is intimately related to the transfer of heat through the cutaneous layers. The energy transfer measurements are still in progress.

Body content of radium in individuals with no known exposure. J. B. HURSH and A. A. GATES (by invitation). *Dept. of Radiation Biology, Univ. of Rochester, Rochester, N. Y.* The commonly accepted maximum permissible level of radium in the body is 1×10^{-7}g. This level is about ten times higher than the lowest radium content known to have produced crippling bone lesions. It is, therefore, noteworthy that A. Krebs, working in a German laboratory, measured the radium body content of eighteen individuals who died with no known radium exposure and found a range of body contents from less than 1×10^{-9} to 4×10^{-8} g radium, with an average of 1.4×10^{-8} g. If these findings have a general application, the margin between the 'safe' radium level and the level existing in 'unexposed' individuals is uncomfortably narrow. We have, therefore, repeated Kreb's measurements using crematory material available from local hospitals. Total body cremation was carried out at a temperature of 1600°F for two hours. The total ashes were weighed and ground. After mixing, an aliquot was taken for analysis. The sample was digested and put into clear solution. The radium disintegration product, radon, was allowed to accumulate for a known period of time. Radon gas was then boiled off the solution and the activity measured in an alpha counter. The results gave values from 1.3 to 3.6×10^{-10} g radium per total body with an average of 2.7×10^{-10} g. This is about two per cent of Krebs's average figure and introduces a more generous margin between the 'safe' and 'unexposed' value.

Localization of vital dyes in areas of inflammation. HOWARD C. HOPPS and BARBARA C. REITER (by invitation). *Dept. of Pathology, School of Medicine, Univ. of Oklahoma, Oklahoma City, Okla.* To elucidate further the mechanisms by which increased capillary permeability occurs in areas of inflammation, the manner of localization of trypan blue in dermal inflammatory lesions has been studied. In all, 58 inflammants have been investigated including such diverse agents as heat (conduction), cold, infra red and ultra violet rays, N-mustard, various cationic and anionic detergents, hyaluronidase, numerous essential oils (cantharidin, methyl salicylate, etc.), pyridine, chloroform, acetone, histamine, leukotaxine, and products of streptococcus pyogenes including intradermal infections as such. Quantitative data have also been secured as to the degree of localization of dye in relation to time and intensity of inflammatory reaction. These observations suggest that localization of such dyes as trypan blue differs qualitatively as well as quantitatively in response to different types of inflammatory stimuli.

Production of 'sickling' in normal red blood cells. RAPHAEL ISAACS. *Michael Reese Hospital, Chicago, Ill.* If a drop of blood is mixed with a drop of concentrated 'fish gelatin' (e.g., Le Page's glue) the red blood cells will take on the sickle shapes at once. This phenomenon is noted in normal human blood, in the blood of animals, even those with nucleated erythrocytes, in sicklemia and in spherocytosis. In an extensive list of diseases tested, the sickling response was the same. The cells are first aligned along the lines of stress in the thick jelly, but later retain the sickle form when thoroughly mixed. If the glue is allowed to dry, the sickling is preserved. In wet preparation, under a cover glass, the cells eventually become spherical, and later hemolyze. Sickling, then, is a property of all red blood cells. In sicklemia and in sickle cell anemia some factor is present which makes the cells sickle under conditions which the normal cell resists.

Methionine and choline in the arrest of dietary cirrhosis of the liver in the rat. ERNEST R. JAFFE (by invitation), ROBERT W. WISSLER (by invitation) and EARL P. BENDITT. *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* An experiment with young albino rats is described in which it was possible to evaluate quantitatively the influence of methionine and choline on the progression of dietary cirrhosis. After a 70-day preparatory period on a low protein, high fat ration, the animals were offered diets containing crystalline amino acid mixtures in place of casein with various quantities of methionine, cystine, and choline for 32 days. When the rats ate 55 to 69 mg. of methionine or 27 to 33 mg. of choline chloride per day, cirrhosis was absent or much less marked than when they con-

sumed about 11 mg. of methionine and no choline. No accentuation of hepatic fibrosis was noted that could be attributed to high levels of cystine, and the combination of cystine plus choline was not as effective in arresting the scarring as methionine and choline. The influence of methionine and choline on the cirrhosis was closely correlated with the ability of these dietary essentials to alter the large droplet fatty change present in rats after 70 days on the basal ration. These substances, perhaps through their lipotropic properties, caused a disappearance of the large globules, and this improvement in the structure of the parenchymal cells may, in part, have delayed the progression of the hepatic fibrosis.

Tocopherol requirements during the rat's menopause. HANS KAUNITZ and C. A. SLANETZ (by invitation). *Depts. of Pathology and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City.* An inbred colony of albino rats was maintained for successive generations on a simplified diet supplemented by 3 mgs. synthetic dl alpha tocopherol (Hoffmann-La Roche) per 100-gm diet. When the females were 3–6 months, mating resulted in 90% pregnancies; at 10–24 months, only 50% gestations (both normal or resorptions) were observed. When the tocopherol supplement was omitted, reducing the daily intake from about 300 to 30 micrograms, 50% pregnancies (nearly always resorptions) occurred when the females were 3–6 months. Those old rats on the 'complete' (3 mgs tocopherol) diets, which had become sterile, were given 30–60 mg. tocopherol weekly, resulting in 16 pregnancies among 37 rats. More than half of the females on the 'complete' diet gradually developed a condition which could be termed menopause because it was associated with irregularities and finally with absence of the estrus cycle as well as with sterility. This is not necessarily a consequence of 'natural aging' because tocopherol supplements restored the estrus cycle and the fertility in a high percentage of the animals. The tocopherol requirements of the rat essential for the development of either a normal or a resorptive gestation increase steeply with age. In rats of over 1 year, they are roughly 10 times higher than at 3–6 months. This is one of the causes for the occurrence of menopause on a 'normal' diet.

Level of serum mucoprotein as indicator of disease activity in rheumatic fever. V. C. KELLEY and R. A. GOOD (introduced by IRVINE MCQUARRIE). *Dept. of Pediatrics, Univ. of Minnesota, Minneapolis, Minn.* Winzler and co-workers, who determined the mucoprotein content of the serum in adult human subjects in health and in various disease states, demonstrated elevations of the levels of these proteins in malignancy, in certain acute infections, and in tuberculosis. Since they found a constant ratio to exist between total nitrogen, carbohydrate and tyrosine in the mucoprotein fraction, they used the relatively simple determination of mucoprotein tyrosine as an indicator of mucoprotein level. Using their methods modified to permit employment of the Evelyn photoelectric colorimeter, we determined the serum mucoprotein levels in 50 normal children and in a much larger number of children suffering from a variety of disease conditions, including rheumatic fever. Our values for normal children of different ages were in close agreement with those found in normal adults by these authors and by ourselves as well. The most notable feature of our data on sick children was the abnormally high mucoprotein levels found in a series of patients with rheumatic fever in its active phase. In comparison a similar series of rheumatic patients observed during the inactive or quiescent phase and a few patients with apparently active chorea minor showed entirely normal values. Serial determinations of the serum mucoprotein levels in a smaller group of rheumatic fever patients, followed throughout the cycle of active and inactive periods, confirmed the relationship. The degree of elevation of serum mucoprotein was found to be correlated with the state of rheumatic activity as determined by clinical manifestations and by erythrocyte sedimentation rate.

Sustained elevation of blood cholesterol and phospholipid levels in rabbits given detergents intravenously. AARON KELLNER, JAMES W. CORRELL, and ANTHONY T. LADD (introduced by JOHN G. KIDD). *Dept. of Pathology, New York Hospital, Cornell Medical Center, New York City.* Sustained elevations of blood cholesterol and phospholipid were produced in rabbits maintained on a normal diet by the intravenous injection of the detergents Tween 80 and Triton A-20. A single injection of Tween 80 caused an increase in blood cholesterol and phospholipid to two to three times the baseline level, the values reaching a peak in 6 to 12 hours and returning to normal in 24 to 48 hours. Repeated injections of Tween 80 resulted in a sustained hypercholesterolemia with blood levels 5 to 15 times normal, and a parallel and somewhat greater increase in blood phospholipid levels. A single injection of Triton A-20 caused an increase in blood cholesterol and phospholipid levels to more than 5 times normal, with a peak at 2 days after injection and a return to normal after 5 to 12 days. A sustained elevation of blood cholesterol and phospholipid was maintained in 12 rabbits for 10 weeks by twice-weekly injections of Triton A-20. In most animals the blood serum became opalescent and then milky within a few hours after injection and cleared when the injections were stopped. Whether the increase in blood lipid depends on the actual presence of the detergent in the circulation, or on other factors, remains to be determined.

Fate of cancer cells implanted in susceptible and resistant hosts. JOHN G. KIDD and HELENE W. TOOLAN (by invitation). *Dept. of Pathology, The New York Hospital, Cornell Medical Center, New York City.* The cells of two cancers—a mammary carcinoma and a lymphosarcoma—always grew progressively when implanted in the subcutaneous tissues of C3H mice of the sort in which they had originated, causing huge tumors that brought about death of the hosts within a few weeks after implantation. The cancer cells grew equally well for a time upon implantation in mice of the 'A' strain, but after developing into visible and palpable growths that reached 1–3 cm. in diameter within 8 to 14 days, they regressed abruptly in the alien 'A' hosts, disappearing completely by the 10th to 16th days. The 'A' mice in which such tumors had regressed proved solidly 'immune' to re-implantation, no visible or palpable nodules forming after the subcutaneous implantation of large numbers of tumor cells. To learn more about the phenomena of resistance and immunity just described, a study was made of the cytological and histological changes in and around the cancer cells of regressing tumors, and those implanted in immune hosts. These findings were correlated with the viability of the cells as determined by transplantation tests. In many experiments, transplantable tumor cells were procured from regressing growths that had all but disappeared and at a time when only a few malignant cells remained, imbedded in a great mass of reactive host elements. The tumor cells likewise proved transplantable after a sojourn of 3 to 5 days or longer in the subcutaneous tissue of immune hosts.

Cardiac hypertrophy, an immediate response to Starling's Law of increased energy output of the heart. CECIL A. KRAKOWER and HELEN E. HEINO (by invitation). *Univ. of Illinois, College of Medicine, Chicago, Ill.* The accepted mechanism for increasing the energy output of the heart per stroke by lengthening of the muscle fibers of the heart and that which underlies cardiac hypertrophy is the same. It has not been established experimentally, how soon cardiac hypertrophy sets in, once the energy output of the heart per stroke is increased. The speed of cardiac hypertrophy was studied in chicks standardized as to breed, age, sex, and body weight. They were fed a 6% NaCl food mixture over a period of nine hours. Food and water were then withdrawn. Observations on changes in cardiac mass were made at the end of 24 hours. It is demonstrated that in repeated trials, an increase of 7% or more in the heart weight-body weight ratio is obtained within a 24 hour period, representing an absolute increase in intracellular water, in fat-free dry substance and total nitrogen of the heart. The ratios of fat-free dry substance and nitrogen of heart to carcass were likewise increased. It is concluded that cardiac hypertrophy is an immediate response to an increased energy output of the heart per stroke. It follows that cardiac mass in the normal individual is not stable but fluctuates with shifts in the work of the heart per beat beyond that which is customary. It will be demonstrated that a sound myocardium, responds to a given load with maximum hypertrophy within a few days, in the present experiments 20 plus % in 9 days.

Intravenous detergents in experimental atherosclerosis, with special reference to the possible role of phospholipids. ANTHONY T. LADD, AARON KELLNER, and JAMES W. CORRELL (introduced by JOHN G. KIDD). *Dept. of Pathology, New York Hospital, Cornell Medical Center, New York.* Rabbits were maintained on a normal diet without added cholesterol and were given twice daily intravenous injections of Tween 80 for 8 to 14 weeks. Controls were fed cholesterol but given no Tween 80. Despite the fact that the animals given detergent had considerably higher mean cholesterol levels than the controls, they had significantly less atherosclerosis. In the animals receiving detergent, the blood phospholipid levels were as high or higher than the corresponding cholesterol levels, whereas in the controls, the phospholipid levels were about half the cholesterol levels. Experimental atherosclerosis produced in rabbits by cholesterol feeding is usually accompanied by a great increase in blood cholesterol and only a slight increase in phospholipid. Other experiments in which rabbits were fed cholesterol and also given intravenous detergents indicate that despite a sustained hypercholesterolemia, the incidence and severity of atherosclerosis is decreased if the blood phospholipid is elevated concomitantly with the cholesterol. Intravenous detergents were ineffective in the resorption of atherosclerosis previously produced by cholesterol feeding. It seems probable that the cholesterol-phospholipid ratio is an important factor in experimental atherosclerosis, and that increased blood phospholipids may modify or prevent the development of atherosclerosis.

Observations on lung pathology following the inhalation of radioactive cerium. HERMANN LISCO and M. P. FINKEL (by invitation). *Argonne National Laby., Chicago, Ill.* Radioactive cerium (Ce^{144}) is one of the rare earths which is formed in the fission of uranium and of plutonium. It is a beta emitter (maximum energy 0.348 Mev) and it has a half-life of 275 days. Ce^{144} was administered to rats as an aerosol (CeO_2) in doses ranging from 3.2 to 200 μc per animal. Although the material was deposited generally in all lobes of the lungs, its distribution was not uniform. Radioautographs showed small and large, irregularly distributed foci. A considerable portion of the inhaled cerium

remained in the lungs. However, in addition to excretion, some translocation occurred primarily to the liver, kidneys and skeleton. Histologic examinations were made of the lungs of most of the animals, especially those surviving beyond 200 days. The lesions observed will be discussed and illustrated. Particular emphasis will be placed on the occurrence of metaplastic changes in the bronchial epithelium and on malignant tumors arising therefrom.

Varying genetic resistance of rabbits to quantitative inhalation of human tubercle bacilli. MAX B. LURIE, SAMUEL ABRAMSON and A. G. HEPPLESTON. *Henry Phipps Institute, Univ. of Pennsylvania, Philadelphia, Penna.* The fundamental variant tuberculosis caused by virulent bovine tubercle bacilli in highly inbred rabbits of varying genetic resistance to tuberculosis is the degree of localization of disease at the portal of entry and the chronicity of ensuing disease. Rarely, is the bovine infection completely overcome even by the most resistant family. Bovine disease in rabbits of different genetic resistance to infection differs only in degree, not in kind. It has been demonstrated that if genetically susceptible and resistant rabbits simultaneously inhale from 100 to 2000 virulent tubercle bacilli, human type, resistant rabbits overcome the infection completely so that at the end of 2 to 5 months after exposure the lungs are entirely free of any gross tuberculosis in 80% of the individuals, while in 90% of the susceptible animals there is a disease of varying extent often involving the greater part of the lung parenchyma. Thus, not only can the time necessary for determining resistance be greatly shortened, but the distinction between genetically resistant and susceptible rabbits becomes an all or none difference in the great majority of individuals. There is evidence that genetically resistant animals have an innate capacity to destroy or inhibit growth of inhaled human type tubercle bacilli more effectively than susceptible rabbits. Furthermore, and perhaps as a consequence of this differing capacity, genetically resistant rabbits tend to develop allergic sensitivity to tuberculin more rapidly than susceptible rabbits under these conditions.

Studies on the pathogenesis of experimental necrotizing arteritis. J. H. MCCORMICK, JR. (by invitation) and RUSSELL L. HOLMAN. *Dept. of Pathology, Louisiana State Univ. School of Medicine, New Orleans, La.* Previous studies from this laboratory have shown that acute necrotizing arteritis can be produced with regularity in dogs by feeding them a specified high fat diet for a period of 8 weeks or longer then sacrificing them through renal damage. To determine whether damage to tissues and organs other than the kidney would precipitate the lesions in properly fed dogs, 4 such animals were subjected to light chloroform anesthesia for periods totalling 9 to 28 hours in 6 to 20 days. None of these 4 dogs showed either gross or microscopic lesions in the arterial system. Another group of 3 properly fed dogs were subjected to severe inflammatory reactions, one by means of sterile abscesses induced with turpentine and 2 by bacterial infections. None of these dogs showed arterial lesions. These experiments tend to emphasize the importance of the kidney in the pathogenesis of necrotizing arteritis, and suggest the possibility that the proximal convoluted tubules elaborate a substance that is necessary for the proper utilization of certain lipid substances.

Influence of alloxan diabetes of cholesterol atherosclerosis in the rabbit. HENRY C. MCGILL (by invitation), LOUIS E. PARRISH (by invitation) and RUSSELL L. HOLMAN. *Dept. of Pathology, Louisiana State Univ. School of Medicine, New Orleans, La.* Nine control rabbits fed Rockland rabbit diet with 0.3–.5 gm. of cholesterol in olive oil per day were sacrificed after intervals of 38 to 72 days on this diet. There was an average gain in body weight of 38% and an approximate seven-fold increase in total blood cholesterol. All 6 of these animals sacrificed beyond the 48th day showed gross or microscopic atheroma in the aorta and elsewhere; and the degree of these lesions increased with time. Eight rabbits of similar age and original body weight were injected two or more times with alloxan (180 to 385 mg/kg. total) and after a satisfactory diabetic state was established they were fed the same diet and cholesterol in oil as the control animals. These diabetic rabbits were sacrificed after intervals of 18 to 98 days. Six of the 8 rabbits lost weight but 2 of them showed weight gains comparable to the control rabbits. Despite a tenfold increase in the blood cholesterol level in this group only the rabbit fed for 98 days (which lost 10% in body weight) showed microscopic arterial lesions. Three rabbits injected with alloxan failed to develop sustained diabetes and had lesions comparable to the controls when sacrificed at 40, 52, and 69 days respectively. Contrary to anticipation these results indicated that the diabetic state induced by alloxan retards in both time and degree the development of cholesterol atherosclerosis in the rabbit. These experiments confirm the results of Duff and his co-workers.

Experimental papillary necrosis of the kidney. EMANUEL E. MANDEL (by invitation) and HANS POPPER. *Dept. of Pathology, Northwestern Univ. Medical School, the Hektoen Institute for Medical Research of the Cook County Hospital and the U. S. Public Health Service, Chicago, Ill.* Necrosis of the renal medulla has been reported in old European papers to be produced in experimental animals by the administration of vinylamine. In order to study pathogenesis and functional changes incident to such a lesion, vinylamine (ethyleneimine) was

given to 20 rabbits in single or divided doses of 0.005 to 0.03 gm/kg. body weight. On the first day following injection of a lethal dose, excessive hyperemia of all medullary vessels occurred, associated with edema and hemorrhage particularly around the bundles of vasa recta at the corticomedullary junction. The cortex showed striking ischemia except for distended veins. Necrosis of epithelium of the proximal convoluted tubules was often present. In later stages (3–14 days after intoxication), when the cortical epithelium appeared either almost or completely normal or showed evidence of regeneration, conglutination thrombi of erythrocytes were seen in engorged medullary vessels. There were well circumscribed foci of ischemic necrosis of varying size in the papillae. These animals did not develop anuria but showed albuminuria, hematuria and progressive azotemia with almost complete abolishment of renal concentration power as indicated by a urine/plasma quotient of close to unity. In animals surviving the intoxication more than 2 weeks, fibrous scars were found in the papillae and also replacing the vasa recta bundles. These findings appear to throw light both upon a vascular pathogenesis of human papillary necrosis and upon the renal functional impairment occurring in predominantly medullary injury. They, furthermore, illustrate the duality of renal circulation (Trueta).

Hypoprothrombinemia due to loss of intestinal lymph. J. D. MANN (by invitation), F. D. MANN (by invitation) and J. L. BOLLMAN. *Mayo Foundation, Rochester, Minn.* When all of the intestinal lymph of the rat was drained externally, marked hypoprothrombinemia, as determined by the two-stage method, developed rapidly, usually within 24 hours. If adequate amounts of vitamin K were administered parenterally a normal level of prothrombin was maintained, despite loss of lymph and even of considerable amounts of blood as well. Transfusion of twice the animal's normal plasma volume did not maintain normal prothrombin while lymph was lost. Under the conditions of these experiments it appeared that vitamin K was absorbed practically exclusively through the lymph and very little of it was stored, while the turnover of prothrombin was extremely rapid.

An additional thermostable component in the mechanism of leukocytosis with inflammation. VALY MENKIN. *Agnes Barr Chase Foundation for Cancer Research, Temple Univ. School of Medicine, Philadelphia, Penna.* The leukocytosis-promoting factor (LPF), described previously, offers in part a reasonable explanation for the mechanism of leukocytosis with inflammation. Nevertheless, an acute experimental inflammation is frequently associated with a leukocytosis of such a high level that it cannot always be explained by liberation of LPF. This is especially seen in the later stages of an inflammatory state when the exudate tends to be at an acid *p*H, and from which the LPF is not always readily recovered. It has been observed that the non-injurious pyrogenic factor, pyrexin, frequently recovered from acid exudates, induces not only an elevation in temperature, but also an initial leukopenia due to the presence of the leukopenic factor, described previously. The initial leukopenia is subsequently followed by a marked leukocytosis. Boiling pyrexin fails to inactivate this leukocytosis-promoting component. This latter is evidently thermostable. Its effect is very rapid. Even one hour following administration of boiled pyrexin, the effect of the thermostable component of the leukocytosis-promoting factor is manifested by the discharge of immature polymorphonuclear leukocytes. This thermostable component is found in boiled whole acid exudate. In alkaline exudates it is less pronounced, and in such boiled exudates only the discharge of immature leukocytes may be encountered. This thermostable component has been observed in the inflamed pleural cavity of dogs, inflammation being induced with either turpentine or croton oil in olive oil and in the inflamed pleura of guinea pigs with the reaction caused with croton oil in olive oil. The mechanism of leukocytosis with inflammation seems referable to two components: a thermolabile component distributed between the α 1 and α 2 globulins of exudate and a thermostable component associated with pyrexin in the euglobulin fraction found particularly in acid exudates.

Effects of thyroid feeding on histologically normal islets of rats receiving small doses of alloxan. DAVID W. MOLANDER (by invitation) and ARTHUR KIRSCHBAUM. *Dept. of Anatomy, Univ. of Minnesota Medical School, Minneapolis, Minn.* Impairment in glucose tolerance appeared in rats receiving 4 intravenous injections of 20 mg/kg. of alloxan on alternate days. Fasting blood sugar levels were not elevated. Microscopically the pancreatic islets were normal. Rats receiving 10 injections of the same dose of alloxan showed glucose tolerances similar to those found in animals receiving four doses. Beta cell degeneration was present, however, in the islets. Neither glucosuria, polyuria, nor polydipsia occurred in either the 4 or 10 dose animals. Extensive beta cell degeneration was observed in rats receiving one dose of 30 mg/kg. These rats exhibited persistent hyperglycemia and glucosuria, and decidedly altered glucose tolerance. Growth was stunted, whereas it was normal in animals receiving the 4 or 10 injections. If thyroid (0.1 gm/kg. daily by stomach tube for one month) was fed to rats following the 4 doses of alloxan, glucosuria and fasting hyperglycemia occurred. This condition persisted until the rats were autopsied one month after the last feeding. If rats were adrenalectomized preceding the administra-

tion of thyroid, clinical diabetes did not appear. The islets of thyroid-fed alloxanized rats revealed a reduction in the number of beta cells. In the rats that were adrenalectomized preceding thyroid treatment, and in which clinical diabetes did not appear, no changes in the islets were observed. Thus, by functionally altering the beta cells with small repeated doses of alloxan, the added metabolic strain imposed on islet tissue by thyroid feeding was detectable. Lower blood sugar (reduced gluconeogenesis) following adrenalectomy was probably important in preventing beta cell damage.

Induction of hepatic carcinoma in rats by 4-dimethylaminobenzene-1-azo-1-naphthalene. A. S. MULAY and H. I. FIRMINGER (introduced by HAROLD L. STEWART). *National Cancer Institute, Bethesda, Md.* Azonaphthalene (4-dimethylaminobenzene-1-azo-1-naphthalene) was fed to 80 Osborn-Mendel rats, in the dose of 0.075 gm/100 gm. of diet for 8 months. The dye was suspended in corn oil, and this in turn was incorporated in a basic diet consisting of casein, sugar, vitamin mix, and salt mix. Following the azonaphthalene diet period, the animals were returned to their normal laboratory diet of Purina Chow pellets. They were examined at regular intervals, and the date of the first palpable tumor was noted. The clinical diagnosis of the tumor was confirmed at autopsy by microscopic examination in all cases. Fifty-six animals have been autopsied to date and 11 tumors have been found, an incidence of 19.5%. In this series, the first tumor appeared 8 months after the start of the experiment. If this interval is taken as the latent period for tumor induction by this compound, and the percentage of tumor incidence is then recalculated on the basis of those animals surviving the incubation period, the incidence of the tumor increases to 31%. The changes in the liver were similar to those induced by paradimethylaminoazobenzene. Microscopic examination showed fatty change, fibrosis, bile duct proliferation, cirrhosis, cyst formation, and carcinoma. Most of the tumors were hepatic cell carcinomas. They infiltrated the adjacent tissues, and metastasized to the peritoneum, mesentery, and the lungs.

Renal cytoplasmic inclusions following the administration of large doses of streptomycin. CHARLES W. MUSHETT and ROBERT B. STEBBINS (by invitation). *Merck Institute for Therapeutic Research, Rahway, N. J.* Repeated subcutaneous injection of streptomycin either as the sulfate or the calcium chloride complex has resulted in the appearance of acidophilic 'colloid-like' droplets in the cytoplasm of the renal tubular epithelium of rats and dogs. The droplets, varying in size from small granules to relatively large globular bodies, have been seen within one week when daily injections of drug (400 mg. base/kg. body weight) were given. After discontinuance of treatment in rats dosed daily for 21 days, it was observed that the droplets became smaller in size and had disappeared within 4 weeks. With hematoxylin-eosin stain the inclusions have a deep pink color; some give a positive reaction for hemoglobin by the Dunn-Thompson technique; they are not visualized by the usual fat staining methods. Salts of dihydrostreptomycin, the reduced form of streptomycin, also produced these inclusions. The feeding of natural stock diets, highly purified diets or diets containing 5% liver powder had no influence on the appearance of the inclusions. No abnormalities in renal function were observed in dogs whose kidneys were found to contain the 'colloid-like' cytoplasmic inclusions. The nature of the renal inclusions observed after treatment with streptomycin or dihydrostreptomycin is as yet undetermined.

Comparison of different lots of bacitracin for nephrotoxicity to rats and mice. A. A. NELSON and E. C. HAGAN (by invitation). *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* Five commercial lots of bacitracin, suspected of varying in their degree of nephrotoxicity when given parenterally in man, were studied. Microscopic sections were made from 140 animals. All 5 lots caused severe kidney damage when 5000 U/kg. in 2% solution were injected intraperitoneally twice a day in mice for 3 days. Variation in kidney damage between lots was no greater than that between different batches of mice when the same lot was used. The basic lesion was necrosis and sloughing of the convoluted tubular epithelium, evident as early as 1 hour after a single injection. Morphological effect paralleled dosage, and our impression was that when a certain concentration of bacitracin occurred in the tubule there was a direct toxic effect on the epithelium. Saline solutions were slightly less toxic than water solutions. In a general way the histological appearance was similar to that described by Scudi et al. (*Proc. Soc. Exper. Biol. & Med.* 64: 503 and 66: 558, 1947). In our rats, however, lesions were not 'insignificant'; with equal dosage, rats showed even greater kidney lesions than did mice. Nephrotoxicity in rats from intramuscular administration was as great as from intraperitoneal; in mice it was much less. Intramuscularly, local inflammatory reaction was severe in rats and less so in mice; intraperitoneally, local reaction was slight. Organs other than kidney showed infrequent or little effect. Limited study of repair showed that in mouse kidneys the bacitracin picture persisted for about 15 days, changing after 30 days into one of a nonspecific nephritis.

Bone deposition of uranium. W. F. NEUMAN, M. W. NEUMAN, E. R. MAIN and B. J. MULRYAN

(introduced by F. S. ROBSCHEIT-ROBBINS). *Dept. of Radiation Biology, School of Medicine & Dentistry, Univ. of Rochester, Rochester, N. Y.* Previous studies have indicated that the major site of long-term storage of uranium injected into animals was the skeleton. Once deposited, the uranium was mobilized very slowly and underwent very little redistribution within the skeleton itself. A series of studies *in vitro* was conducted on the mechanism of this skeletal deposition. The results of these experiments indicate that uranyl ions undergo exchange with calcium ions in the surfaces of the bone, mineral crystals. In this exchange, uranium apparently combines with two adjacent phosphate groups in the mineral surface to form a but slightly dissociated complex.

Reticulocyte ripening and nutrition in anemic dogs. A. NIZET (introduced by F. S. ROBSCHEIT-ROBBINS). *Univ. of Rochester School of Medicine and Dentistry, Dept. of Pathology, Rochester, N. Y.* The ripening time of the reticulocytes has been measured by a technique previously described in anemic dogs and doubly depleted dogs (anemia and hypoproteinemia). Anemia is produced by blood withdrawal, double depletion by blood withdrawal plus a non-protein containing diet. The progressive disappearance of the various reticulocyte groups is studied in heparinized blood samples at body temperature. In the anemic dogs receiving a basal diet containing proteins the ripening time is the same as in non-anemic dogs, regardless of the supplementary diet factors. In the anemic hypoproteinemic dogs (double depletion) in contrast receiving a non-protein basal diet the ripening time depends upon the supplementary diet factors. With the non-protein diet alone in these doubly depleted animals the ripening is almost completely blocked. Following administration of poor supplementary diet factors (low hemoglobin regeneration) the ripening time is greatly prolonged. With an optimum supplementary diet (maximum hemoglobin regeneration) the ripening time is normal. The rate of hemoglobin regeneration *in vivo* and the ripening time of the young erythrocytes in vitro coincide very well.

Histopathology of the liver following histamine infusion. W. G. NOTHACKER and RALPH W. BRAUER (introduced by RUSSELL L. HOLMAN). *Depts. of Pathology and Pharmacology, Louisiana State Univ. School of Medicine, New Orleans, La.* When histamine diphosphate was infused continuously into the portal vein of a dog under sodium pentabarbital anesthesia, at such a rate as to depress the mean arterial blood pressure to approximately 50% of the initial level, there was a fall in the portal venous pressure, an increase in lymph flow from the liver, and a reduction of the rate of bromsulphthalin removal from the blood plasma. In animals sacrificed after 4 hours or more of such infusion, the liver was enlarged, and mottled red and red brown with prominent portal areas. Upon histological examination these livers showed neutrophils and lymphocytes about the portal veins, dilatation of portal lymphatics, leukostasis of neutrophils in the sinusoids, constriction of central veins and occasional hemorrhages into the portal areas. When histamine infusion was stopped, at any time during the experiments, the mean arterial blood pressure regularly rose to the initial level within ten minutes or less and the animal survived. Control animals subjected to the same experimental procedures but infused with normal saline showed none of the above physiological and anatomical changes. These studies indicate that histamine in sublethal doses is capable of producing definite liver injury.

Replacement of a portion of common bile duct by a segment of uterine horn. CHARLES M. O'LEARY (by invitation), WILLIAM T. SNODDY (by invitation), and BÉLA HALPERT. *Depts. of Surgery and Pathology, Univ. of Oklahoma School of Medicine, Oklahoma City.* The right uterine horn of the dog was resected with its blood supply from the ovarian artery left intact. The common bile duct was severed near its junction with the duodenum and the duodenal end ligated. The proximal end of the common bile duct was then telescoped into the proximal end of the uterine horn while the distal end of the horn was pulled through a longitudinal slit made in the duodenal wall about 2 cm. anterior and medial to the choledochoduodenal junction. Both ends of the uterine horn were secured in place by cotton sutures. Of 20 dogs surviving the operation 12 lived for 3 to 21 days, the rest were sacrificed between 4 and 27 weeks following the operation. Detailed morphologic studies are in progress to determine the mechanism by which the continuity of the flow of bile into the duodenum was maintained and the effects of the replacement of the terminal segment of the common bile duct on the biliary system.

Restoration of estrous cycle by alpha tocopherol in old rats. S. Y. P'AN, HANS KAUNITZ, and C. A. SLANETZ (by invitation). *Depts. of Pharmacology Pathology, and Animal Care, College of Physicians and Surgeons, Columbia Univ., New York City.* An inbred colony of albino rats was maintained for successive generations on a simplified diet containing 3 mg. (synthetic alpha tocopherol (Hoffmann-La Roche) per 100 gm.; this permitted a daily intake of approximately 300 micrograms of tocopherol as usually provided for laboratory rats. Daily vaginal smears were made on 64 females of 10–20 months for 4 weeks or more. In 16, the estrous cycle was of 4–5 days duration; in 34, it lasted more than 8 days; and in 15 of this latter group, it lasted longer than 17 days in 80% and more than 12 days in 20%. The latter 15 females received 30-

60 mgs alpha tocopherol weekly by mouth. In 3 rats, cycles of 4–5 days and in 5, cycles of 6–8 days were observed within 2 weeks after treatment was begun. There was no change in the remaining 7 animals. It is improbable that the shortening of the cycle was due to spontaneous changes because 4 untreated controls with cycles of 9–12.5 days were smeared during the period of observation with lengthening of the cycles. It seems evident that, in a high percentage of old rats maintained on a diet with 'normal' tocopherol supplement, lengthened estrous cycles may occur which can frequently be shortened by additional alpha tocopherol.

Blood cholesterol value of normal men in relation to their basal metabolic rate. ANNIE LAURIE PEELER (by invitation), OPAL E. HEPLER, VELMA MILLER KINNEY (by invitation), LILLIAN E. CISLER (by invitation), and FREDERIC T. JUNG (by invitation). *Depts. of Pathology and Physiology, Northwestern Univ. Medical School and Passavant Memorial Hospital, Chicago, Ill.* Because of the large range of normal cholesterol values the correlation was determined between the blood cholesterol of normal men and their basal metabolic rates. Bloor's method adapted to the Evelyn photoelectric colorimeter was used to determine cholesterol in serum. Values for 73 male medical students ranged from 131–303 mg/100 ml. of serum with a mean of 189 mg. and a standard deviation of ±33. Four fell below 150 and 6 above 250, leaving 86% with a range from 150 to 250 mg. The metabolism tests were performed under basal conditions using a Benedict-Roth machine. The results presented are the percentages above and below normal using the Boothby and Sandiford modification of Dubois standards. Values for 73 men ranged from −24.9 to +10% with a mean of −9.5% and a standard deviation of ± 7.2. No correlation was found between these basal metabolic rates and cholesterol values as shown by the correlation coefficient of − 0.10. Both cholesterol and basal metabolic rates were repeated on 40 of the men after a 38-day period which included 2 weeks of vacation. The coefficient of correlation between the first and second cholesterol values was +0.73 which indicates that the blood cholesterol is maintained at a fairly stable level. This was also true, to a lesser extent, of the basal metabolic rates which gave a correlation coefficient of +0.65 between the first and second periods.

Quantitative correlation between results of hepatic tests and morphologic liver cell damage. HANS POPPER, FREDERICK STEIGMANN and PAUL B. SZANTO (by invitation). *Hektoen Inst. for Medical Research of the Cook County Hospital, the Dept. of Pathology of Northwestern Univ. Medical School and the Dept. of Internal Medicine of Univ. of Illinois College of Medicine, Chicago, Ill.* The degree of liver cell damage was graded 0 to 3 plus in 257 liver biopsy specimens obtained from patients with various diseases, mostly hepatobiliary in nature. The results were compared with those of a series of hepatic tests performed simultaneously with the biopsy. In confirmation of previous studies concerning correlation of presence or absence of pathologic findings with results of hepatic tests, it was noted that the higher the grade of liver cell damage in a group, the higher the average of the cephalin flocculation, thymol turbidity, serum globulin, urinary urobilinogen, alkaline phosphatase, prompt reacting and total bilirubin, and the lower the albumin, albumin/globulin ratio and prothrombin time. No parallelism was found with total serum protein, total cholesterol, nonprotein nitrogen, sedimentation rate and fecal urobilinogen. The described parallelism was not necessarily present in individual cases. Moreover, in extrahepatic biliary obstruction without secondary infection, cephalin flocculation and thymol turbidity did not parallel the degree of liver cell damage, the cephalin flocculation being negative and the thymol turbidity only slightly elevated even with severe damage. Contrarily, the results of these two tests paralleled the degree of liver cell damage if bacterial infection complicated extrahepatic obstruction. To exclude liver cell damage, cephalin flocculation and urinary urobilinogen appeared the most valuable tests. The number of tests with abnormal results paralleled the degree of liver cell damage in the different groups. In general, the frequent exception in individual cases do not contradict a general tendency to a quantitative relation between functional and structural hepatic alterations.

Electron microscopy of clinical marrow aspirates. J. W. REBUCK and H. L. WOODS (introduced by F. W. HARTMAN). *Dept. of Labys., Henry Ford Hospital, Detroit, Mich.* Direct electron microscopy of sternal aspirates was accomplished as follows. After aspiration of the specimen the customary air-dried smears were prepared from a portion for light microscope studies. The remaining material was utilized, with a modification of Schleicher's technic, for electron microscope studies. One or more gross marrow units were selected and affixed to the blunt end of a wooden applicator. Imprints were then made by touching the marrow unit lightly against formvar-covered glass slides. The marrow cells adherent to the surface of the formvar were fixed over osmic acid vapor and washed in distilled water. The specimens were mounted directly by the modified stripping technic which we have previously reported. Electron micrographs of hematocytologic features were thus obtained. Developing neutrophil granules were observed to be more vacuolar than granular in structure. The bizarre nuclear structure of leukemic reticulum cells from a case of reticular myelosis was depicted.

Superimposition of small osmiophilic spherules was noted. Myeloma cells from a case of multiple myeloma were micrographed. A small, extremely dense particulate substance presented in the cytoplasm of such a cell. In one such case complicated by an acquired hemolytic anemia, interesting structural changes were present in erythrocytes individually and in their agglutinated relationships. Upon erythrocytic desiccation in rouleaux-like aggregates, hemoglobin shrinkage resulted in delineation of the isolated corpuscular 'membrane'. The thickness of this human surface ultrastructure was measured as being between 330 and 1000 Å with a mean of about 550 Å.

Effect of antihistamine on the localization of trypan blue in xylene treated areas of skin. R. H. RIGDON. *Laby. of Exptl. Pathology, Medical Branch, Univ. of Texas, Galveston, Texas.* Localization of dye in areas of skin injected with histamine (*J. Lab. and Clin. Med.* 27: 1554, 1942) and in areas of inflammation produced by xylene (*Arch. Surg.* 41: 101, 1940) has been studied. In the latter it was shown that localization and concentration of this dye was determined by the interval between the application of xylene and the injection of the dye. Furthermore, the localization of the dye was not related to the presence of hyperemia. The influence of antihistamine on the hyperemia produced by xylene and the localization and concentration of dye in these areas were studied in the rabbit. Pyrrolidineethylphenothiazine hydrochloride (Pyrrolazate) and N,N-Dimethyl-N′ (alpha-pyridyl)-N′-(alpha-thenyl)-ethylenediamine hydrochloride (Thenylene) were dissolved in saline (10 mg/ml.). The former was given intravenously and the latter intravenously and intraperitoneally in large amounts. The antihistamine was given frequently before the intravenous injection of trypan blue (10 ml. of a 0.5% solution). Xylene was applied to the skin with a cotton applicator at intervals preceding the injection of the dye. Reaction to the xylene was the same in antihistamine treated animals as in the controls. Furthermore, the localization and concentration of trypan blue was the same as in the controls. Trypan blue localized and concentrated at the site of intradermal injection (0.2 ml.) of antihistamine. It is suggested that histamine is not the primary factor responsible for the localization and concentration of trypan blue in areas of inflammation produced by xylene and, furthermore, antihistamine, when injected intradermally in the rabbit, causes a localization and concentration of dye in a manner similar to the intradermal injection of histamine.

Multiple injections of potassium as a test for adrenocortical function, with modifications induced by desoxycorticosterone. THEODORE ROBERTSON (introduced by JOHN G. KIDD). *Dept. of Pathology, New York Hospital—Cornell Medical Center, New York City.* As a means of studying the function of the adrenal cortex a modification has been made of the potassium toxicity test previously employed by others. Four intraperitoneal injections of potassium chloride solution were given at 2-hour intervals to mature rats and mice. Dosage was calculated in milligrams of KCl/10 gm. of body weight. Significant differences were found when the test was applied to normal and adrenalectomized animals. For the L. D. 50 in normal rats was about 4.5 mg/10 gm., and in normal mice was slightly less than 5 mg/10 gm., whereas in adrenalectomized animals given salt and tested on the sixth postoperative day, the L. D. 50 values were approximately 2.0 and 2.6 respectively in the two species. A consistently increased resistance to potassium was obtained by the administration of small amounts of desoxycorticosterone acetate in various ways to normal and adrenalectomized animals. The only variation attributable to sex was found in adrenalectomized rats about 11 weeks old. When tested with 2 mg. KCl/10 gm. there was a 90% mortality in males (18 of 20) and a 15% mortality in females (2 of 13). The results obtained with the potassium toxicity test suggest an ovarian influence on potassium metabolism as an addition to those influences previously recognized. The test has also been applied with interesting results to animals in which a depletion of the zona glomerulosa of the adrenal cortex had been effected by means of pellets containing desoxycorticosterone.

Intradermal reaction to streptococcal thermal antibody and antigen in persons having epidemic respiratory infections. EDWARD C. ROSENOW. *Bacteriological Research, Longview Hospital, Cincinnati, Ohio.* The solutions of thermal antibody and of antigen injected intradermally consisted of the bacteria-free supernatants of saline suspensions of streptococci isolated in studies of respiratory infections. The former after autoclaving suspensions containing twenty billion streptococci/ml for 96 hours and adding an equal volume of saline containing 0.4% phenol; the latter after heating suspensions containing ten billion streptococci/ml at 70°C. for one hour and adding 0.2% phenol. Three hundredths ml. of each and of control solutions were injected into the skin of the anterior aspect of the forearm of 272 persons having respiratory infection and of 483 well persons. Reactions to the 'respiratory' thermal antibody indicating specific antigen in skin or blood and of a streptococcal infection occurred in nearly all persons having respiratory infection. The reactions were largest during the acute stage of the disease and gradually diminished as recovery ensued. In sharp contrast reactions to 'respiratory' antigen indicating antibody were minimal at the onset, in-

creased as recovery occurred and then diminished as antigen disappeared. The incidence of significant reactions to the 'respiratory' antibody indicating antigen in well persons during summer was minimal. It increased in autumn and remained relatively high during winter and spring but was uniformly less in persons having symptoms of respiratory infection. Reaction to the 'respiratory' antigen was absent or least pronounced in well persons remote from respiratory infections and increased with the seasonal incidence. Reactions in well persons to 'poliomyelitic' streptococcal antibody and antigen were in reverse of those to 'respiratory' antibody and antigen.

Contagiousness of coccidioidomycosis. Sol Roy Rosenthal. *Univ. of Illinois, College of Medicine, Chicago, Ill.* Experiments conducted in 3 phases show that coccidioidomycosis is a contagious disease: *1*) Spherule containing exudates from humans or guinea pigs when instilled into the trachea of guinea pigs produced a primary form of the disease very similar to that found in humans. *2*) Spherule containing exudates of humans kept in the refrigerator for periods up to 110 days failed to produce mycelial threads and chlamydospores but retained their spherule forms that were infective to guinea pigs. Spherule containing sputum exposed out of doors to the shade or the sun with or without earth for periods up to 230 days retained their spherule forms, albeit in a certain percentage of cases mycelial forms were found. *3*) In a limited number of cases placing normal guinea pigs in the same cages with animals infected intratracheally with the spherules of coccidioides immitis resulted in infection of the control animals.

Electrocardiograms of normal and malarial infected monkeys. Arthur Ruskin (by invitation) and R. H. Rigdon. *Heart Station and Laby. of Exptl. Pathology, Univ. of Texas, Medical Branch, Galveston, Texas.* Electrocardiograms in 14 normal *M. rhesus* monkeys weighing from 2 to 6 kg. simulated those of normal humans with the following differences. The QRS wave was usually of very low voltage in Lead I. The T wave in Lead I was likewise usually very low. It was flat in 7 monkeys. However, in 1 it was slightly negative on another occasion. The T wave in Lead II was flat in 2 cases, in both slightly negative on other occasions. T4 was flat in 2 animals and slightly negative in one. These findings controvert those of deWaart and Storm. Heart rate varied from 190 to 280, P-R interval from 0.06 to 0.10 sec., QRS from 0.02 to 0.04 sec., Q-T from 0.14 to 0.18 sec., the axis from 50° to 115°. Marked S-T segment depression occurred particularly in Lead II and III in 6 cases. An auricular ectopic beat was the only arrhythmia noted. Electrocardiograms in 12 monkeys infected with *P. knowlesi* did not vary significantly from normal. This is consistent with the absence of specific pathologic lesions in the heart of malarial infected monkeys. T waves in Lead I were flat in 6 of 12 infected monkeys. In 2 of the same group on different days T waves in Lead I were negative; however, both monkeys showed similar findings before infection. In 2 monkeys with severe parasitemia, the Q-T intervals were 0.20 at a ventricular rate of 188, and 0.23 at a rate of 159. One also showed left axis deviation of −48°, another very ill animal had an axis deviation of −40°. With these three exceptions, electrocardiograms in malarial infected monkeys were similar to normals.

X-ray induced developmental abnormalities in the mouse. Liane Brauch Russell (introduced by F. S. Robscheit-Robbins). *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.* In an attempt to determine the effect of penetrating radiation on developing mouse embryos, pregnant females were irradiated at various stages of gestation. Each female was given a single exposure to 250 kvp x-ray total body irradiation, doses ranging from 200–500 r at an intensity level of around 80 r/min. Gestation was timed from the observation of a vaginal plug and the stages examined cover almost the entire range of embryonic life. Highly inbred C57 Black strain females, made pregnant by NB strain males, yielded the genetically uniform F_1 fetal population. Except for a few cases in which the litters were raised, the irradiated fetuses were allowed to develop only to birth (or the expected date of birth), at which time they were carefully examined externally and then processed to give cleared and stained skeletal preparations. Our results indicate that certain structures are sensitive to x-ray effect only during definite periods in development. Dosage-effect relations at particular stages have proved to be interesting. It is hoped that the determination of sensitive stages for each structure, the entire syndrome produced at a particular stage and the order in which certain changes are produced—all considered in conjunction with the known facts of normal development and of hereditary abnormalities—may shed some light both on the nature of the radiation effect and the nature of development.

Prompt and indirect reacting serum bilirubin in jaundice. Fenton Schaffner (by invitation), Hans Popper and Frederick Steigmann. *From the Hektoen Inst. for Medical Research of the Cook County Hospital, the Dept. of Pathology of Northwestern Univ. Medical School, and the Dept. of Internal Medicine of Univ. of Illinois College of Medicine, Chicago, Ill.* Serum bilirubin determinations (1 minute and total) were obtained on 195 jaundiced patients with cirrhosis (152), acute hepatitis (171) and extrahepatic biliary obstruction (99). Results were correlated in 132 instances with liver biopsies. The ratio of prompt reacting to total bili-

rubin followed similar distribution curves with almost half the values lying between 40 and 50%. Too few cases of hemolysis were observed to evaluate statistically but in each instance the ratio of prompt to total bilirubin was below 15%. When the ratio was plotted against total bilirubin, ratios ranged in random fashion between 12 and 73% when the total was below 10 mg/100 cc. When above this, 82.5% of the ratios were between 30 and 50%. As the level rose, the ratio fell. It was below 40% in 65% of the determinations when the total was above 40 mg/100 cc. No relation between ratio and degree of liver damage was noted. The only significance of an elevated prompt bilirubin in the presence of jaundice is its exclusion of hemolysis. This does not support the view that bilirubinemia arises in a different manner in parenchymal and obstructive jaundice. The evidence suggests that indirect bilirubin is changed to prompt reacting in the Kupffer cells from where it is transmitted to the liver cells. Inability of Kupffer cells to accept indirect bilirubin explains the elevated indirect level in non-hemolytic jaundice, while leakage from the Kupffer cells or regurgitation explains the elevated prompt levels.

Preparation and properties of mammalian striated myofibrils. ARMIN F. SCHICK, (by invitation) and GEORGE M. HASS. *Rush Dept. of Pathology, Presbyterian Hospital, and the Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago, Ill.* A physico-chemical method for isolation and purification of myofibrils from mammalian skeletal and cardiac muscle has been devised. Blocks of living muscle were frozen and cut into sections with a microtome. Sections were transferred to a proteolytic enzyme buffer solution (0°C.; *p*H, 7; ionic strength, 0.25). After 30 to 45 minutes, the myofibrils were separated by mechanical agitation. Then, the segregated myofibrils were separated from other components of muscle by centrifugation. The isolated myofibrils obtained in large quantities had characteristic properties. They varied in length and breadth but retained microscopic structural detail, plasticity and birefringence, characteristic of myofibrils in their intracellular location. They were soluble in a slightly alkaline reagent (0.5 N KCl – 0.03 N sodium bicarbonate) giving viscous solutions which displayed birefringence of flow. In buffer solutions with an ionic strength of 0.15, myofibrils dissolved acid to *p*H 4.0 and alkaline to *p*H 10.0. In phosphate buffer solutions with an ionic strength of 0.5, myofibrils dissolved when solutions were alkaline to *p*H 6.3. In phosphate buffer solutions with ionic strength increased from 0.15 to 0.5 by addition of potassium chloride, myofibrils were soluble when solutions were alkaline to *p*H 6.0. Isolated myofibrils, although probably modified by tryptic action and mechanical agitation during isolation at 0°C, exhibited property of contractility when placed in solutions of adenosine triphosphate. Under these conditions, myofibrils isolated from skeletal muscle contracted rapidly so that long fibrillar structures were converted irreversibly into spherical masses.

Electrophoretic findings in normal and abnormal pregnancy. NEVIN S. SCRIMSHAW (by invitation) and ERIC L. ALLING. *School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* Electrophoretic patterns of nearly 200 normal and abnormal pregnant women reveal a tendency for albumin values to be lowered in preeclampsia. In 46 of 52 mild and severe pre-eclamptics, the albumin value was below 2.80. It was below this figure in only 10 of 77 apparently normal pregnancies. The pattern in pregnancy with essential hypertension does not differ from that of normal pregnancy. However, when pre-eclampsia is superimposed on previously existing hypertension, the pattern is that of pre-eclampsia. This is strong evidence that pre-eclampsia and essential hypertension are separate conditions which frequently co-exist during pregnancy. It is usually possible to determine by means of the electrophoretic pattern whether a blood pressure elevation late in pregnancy actually represents pre-eclampsia. In occasional cases, the lowering of albumin has been noted to precede the appearance of clinical signs and symptoms of preeclampsia. The changes in the globulin fractions are less consistent, but the shapes of the peaks as well as the absolute values may be of significance. If a small amount of normal hemoglobin is added to normal plasma before electrophoresis, it migrates with, or just behind, α_2 globulin. In most cases of severe pre-eclampsia, normal hemoglobin so added, migrates more slowly; with β globulin. After delivery the migration soon becomes normal. This phenomena has also been observed in all cord blood examined. It has never been observed in plasma from pregnant women without pre-eclampsia.

Chorionic gonadotrophin as luteotrophin in women. ALBERT SEGALOFF, CORNELIA J. GASKILL (by invitation), WILLIAM STERNBERG (by invitation), and RICHARD L. COPPEDGE (by invitation). *Depts. of Medicine, Physiology and Pathology of Tulane Univ., the Alton Ochsner Medical Foundation, and the Charity Hospital of Louisiana, New Orleans, La.* Five patients were given chorionic gonadotrophin (A.P.L.—Ayerst, McKenna and Harrison) in a dosage of 10,000 I U/day starting after the ovulatory rise in the cycle. Preliminary studies and studies during treatment were made on the urinary excretion of gonadotrophic hormones, 17-ketosteroids, glycogen depositing steroids (cortin) and prolactin. Endometrial biopsies were obtained in the previous cycle and during therapy. Three of the patients required gynecologic surgery and were given the chorionic gona-

dotrophin up to the day of surgery, and the surgical specimen studied. There were no significant changes in the urinary excretion of 17-ketosteroids or cortin. There was a small but consistent rise in the urinary excretion of prolactin, the significance of which at present is obscure, and there was a great increase in the excretion of gonadotrophic hormone due to the urinary spillage of the chorionic gonadotrophin. In all instances, the next menstrual period was delayed significantly, and in only one instance did spontaneous menstruation occur while the medication was being given. The endometrial biopsies and surgical specimens obtained during the administration of chorionic gonadotrophin all showed a definite decidual type of reaction. All of the endometria obtained in the cycle prior to the administration of chorionic gonadotrophin showed typical progestational phase.

Vitamin B_6 deficiency in the Syrian hamster. GREGORY SHWARTZMAN and LOTTE STRAUSS. *Division of Bacteriology, Labys. of The Mount Sinai Hospital, New York City.* Male weanling Syrian hamsters were maintained on a purified diet containing the essential vitamins and biotin but deficient in vitamin B . This led to arrest of growth after a depletion period of 2 to 3 weeks, diminished food and water intake, progressive malnutrition, muscular weakness and changes of the fur. Increased quantities of xanthurenic acid were found in the urine. The deficient animals did not survive beyond 12 to 13 weeks showing loss of fat tissue and marked atrophy of the lymphoid tissues, notably the thymus, even when malnutrition was mild. There was arrest of sexual maturation. Pair-fed controls receiving 50γ pyridoxine HCl daily showed arrest of growth and atrophy of lymphoid tissues, but failed to develop fur changes, muscular weakness, or cachexia. Controls fed ad libitum and receiving daily 50γ of pyridoxine HCl showed good growth and nutrition and normal activity. Their fur was normal and there was no atrophy of the lymphoid tissues. Upon treatment of the deficient animals with daily injections of 50γ of pyridoxine HCl after a depletion period of 9½ weeks, there was in most animals a resumption of appetite and growth, deposition of fat and general return to normal behavior and appearance. Fur changes were repaired within one week of treatment. A single injection of pyridoxine HCl to a deficient animal seemed to bring about similar results, the effect lasting for about two weeks. Although the presence of unsaturated fatty acids in the diet seemed to delay the onset of deficiency symptoms, the addition of corn oil to the diet of animals in an advanced state of depletion did not have any beneficial effect.

Retardation of the reaction time in parathyroidectomized rats. CARL C. SMITH (by invitation) and HERBERT C. STOERK. *Merck Inst. for Therapeutic Research, Rahway, N. J.* 'Reaction time', the time necessary to elicit a response to a painful stimulus, appears to be a complex sum of events involving perception, conduction along sensory and motor neurons and synaptic transmission. Since a state of hyperexcitability of the motor nerve endings is known to exist in parathyroid insufficiency, it was thought that in parathyroidectomized rats reaction times may be measurably shortened. Contrary to expectation it was found that reaction times were significantly prolonged in parathyroidectomized rats. The method of estimating reaction times was that of D'Amour and Smith in which light from a small bulb is focused by a reflector on the blackened tip of a rat's tail. The reaction times of parathyroidectomized rats, ten days after operation, were 10–50% greater than those of controls. Simultaneous measurements as expected demonstrated increased excitability of motor nerves. The prolonged reaction times could be obtained as soon as two days after parathyroidectomy when Na_2HPO_4 (230 mg/kg) was administered orally. The analgesic effect of methadone and the retardation of reaction time due to parathyroid insufficiency appear to be additive.

Effect of malnutrition on the infection of mice with influenza virus. DOUGLAS H. SPRUNT. *Division of Pathology and Bacteriology, Univ. of Tennessee College of Medicine, Memphis, Tenn.* We have previously reported that adult mice, which were fed a diet low in protein but adequate in all other respects, were more resistant to infection with the swine influenza virus than were mice given an adequate diet. The mice were fed the low protein diet for 2 weeks before inoculation with the virus and until the end of the experiment 2 weeks after inoculation. Since this report we have found that this increased resistance to viral infections disappears if the mice are kept on the low protein diet for a longer period of time. It has also been found that the resistance of immature mice cannot be increased by a low protein diet. The immature mice are more susceptible to infection even though they are on the low protein diet. In all these experiments an equal number of mice have been kept without virus in order to see if the malnourished mice are dying of malnutrition. In every instance the diet has proven adequate to support life. At present we do not have sufficient data to explain the above phenomenon but believe it is due to the fact that animals on a low protein diet call upon their reserves of protein and hence are not on a low protein diet until these are depleted. The immature mice we believe are more susceptible because no appreciable reserve exists.

Determination of minimal daily essential amino acid requirements in protein-depleted male albino rats. C. H. STEFFEE (by invitation), R. W. WISSLER (by invitation), E. M. HUMPH-

REYS (by invitation), E. P. BENDITT, R. L. WOOLRIDGE (by invitation), and P. R. CANNON. *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* The response of a group of 24 protein-depleted rats fed a relatively complete amino acid ration was used as the standard for determining the quantitative requirements for each of the nine essential amino acids for adequate regain of weight in ten days. For this purpose 247 protein-depleted rats were used. With different amino acid intakes, weight gains were directly parallel to changes in carcass protein. By varying the intake of each amino acid successively, without altering other dietary components, the requirements for each were ascertained. These requirements represent the minimal quantity of each essential amino acid which, in conjunction with the remainder of the standard ration, produced weight gains equal to those induced by feeding the complete standard ration. Moreover, excellent weight gains followed the feeding of an identical ration, containing only these minimal quantities used have no absolute value, except under these experimental conditions, the proportions of amino acids required in relation to one another may have wide significance. For example, the repleting rat utilizes from one to four times the quantities of essential amino acids needed for maintenance. The requirements for leucine and lysine are greatest, and these amino acids are found in high concentrations in mammalian muscles. It is suggested that the variant results obtained by other investigators with amino acid rations may be due to their use of inadequately proportioned mixtures of amino acids.

Influence of nutrition on plasma vitamin A alcohol. FREDERICK STEIGMANN, HANS POPPER, HATTIE A. DYNIEWICZ (by invitation) and IRENE A. MAXWELL (by invitation). *Hektoen Inst. for Medical Research of the Cook County Hospital; Dept. of Pathology, Northwestern Univ. Medical School; and Dept. of Internal Medicine, Univ. of Illinois College of Medicine, Chicago, Ill.* The plasma vitamin-A level depends upon endogenous factors like diseases (influencing release of vitamin A from the liver and its intestinal absorption) as well as upon nutritional factors. This explains the equivocal correlation between plasma vitamin-A level and nutritional state. Recent experiences indicated that the vitamin-A alcohol level responds more readily than that of vitamin-A ester to changing vitamin-A nutrition. Therefore, vitamin-A alcoho and ester plasma levels were studied in 19 patients with either conditions without known influence upon vitamin-A metabolism (controls) or with liver diseases. They were kept for 1 to 4 weeks on different levels of vitamin-A nutrition (from zero to 20,000 U). These first periods were alternated with others with increased or reduced vitamin-A intake. In controls kept for more than one week on diets not exceeding 1500 U vitamin A, the alcohol level dropped, whereas, the esters revealed irregular variations. After prolonged periods on low intake, rise of alcohol and ester vitamin A might ensue, possibly as result of release from the liver. After complete depletion of over one week, intake of 10,000 U raised the alcohol level consistently without influence upon esters. After a preperiod of 1500 U intake, 3000 U caused a rise in half the cases. Secondary depletion and repletion periods of 1 week duration revealed less consistent results. The curves in patients with liver diseases were erratic. Serial follow up of the plasma vitamin-A alcohol level may, therefore, in normals with certain reservations mirror the status of vitamin A nutrition and possibly permit estimation of the human vitamin A requirement.

Studies of leukocyte production and distribution: factors in plasma and in leukocytes. BERNHARD STEINBERG and RUTH A. MARTIN (by invitation). *Toledo Hospital Institute of Medical Research, Toledo, Ohio.* One of the significant questions in hematology concerns the mechanism which maintains the solid constituents of the blood at a constant definite level. Our investigations (*Proc. Soc. Exp. Biol. & Med.* 56: 50–52, 1944; *J. Immun.* 51: 421–426, 1945; 52: 71–75, 1946; 53: 137–141, 1946) of the immunochemical characteristics of various types of leukocytes of variable ages and physical states suggested the possibility of the presence of regulating substances within the cells. Normal and leukemic plasma with and without contact with normal or leukemic cells as well as leukocytes only were injected into rabbits. The animals were either normal or prepared by injections of benzene and extracts acting on the marrow. Studies were made of marrow, other organs and of the peripheral cell content. The tissues as well as the blood gave indications that intact leukocytes contain a substance which is secreted into plasma and which acts on the marrow to inhibit the expulsion of cells. Degenerating leukocytes, on the other hand, secrete a substance which accelerates cell expulsion from the marrow. Effect of various temperatures on the active substances were determined. There is suggestive evidence that these substances are of the nature of enzymes. Correlations were made with other materials such as milk, which apparently induce a change in the peripheral leukocyte picture by an increase in cell expulsion.

Influence of chorionic gonadotrophin on the hilus cells of the human ovary. WILLIAM H. STERNBERG (by invitation), ALBERT SEGALOFF, and CORNELIA J. GASKILL (by invitation). *Depts. of Pathology and Medicine, Tulane Univ., the Alton Ochsner Medical Foundation and the Charity Hospital, New Orleans, La.* The hilus and mesovarium of the human ovary normally contain nests of cells morphologically identical with Leydig cells. They

contain lipids, acidophilic granules, lipochrome pigment and crystalloids of Reinke as do the interstitial cells of the testis. They are intimately associated with non-myelinated nerves and vascular spaces. During pregnancy and at the menopause they are especially conspicuous. We have recently studied 3 instances of tumor and 3 of hyperplasia of these cells all associated with masculinizing syndromes. Five regularly menstruating patients were treated with chorionic gonadotrophin and their ovaries examined histologically. The dosage was 10,000 U of chorionic gonadotrophin daily to each for periods of 12 to 19 days following ovulation. This dosage usually delays menstruation and produces a decidual reaction. Morphologic changes were noted in the ovarian hilus cells. The cells were present in larger than average numbers, and the cytoplasm more granular and acidophilic. In one case mitoses were present, although mitoses are not ordinarily seen either in hilus cells or in testicular Leydig cells. In 3 cases focal areas of degeneration were noted, including pyknosis, clumping of nuclei, and loss of cell outlines. Similar changes were noted in a case of chorionepithelioma of the uterus, and a teratoma of the ovary in which urinary gonadotrophin was elevated. The findings suggest that ovarian hilus cells are responsive to chorionic gonadotrophin. It is likely that they are one of the sources of androgen in the human female.

Mechanical and osmotic fragility in relation to age of dog erythrocytes tagged with radio-iron. W. B. STEWART, J. M. STEWART, M. J. IZZO and L. E. YOUNG (introduced by C. L. YUILE). *Depts. of Pathology and Medicine, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* The mechanical and osmotic fragilities of dog erythrocytes of known age tagged with radio active iron were measured and compared with the fragilities of the total cell population. Under the stimulation of severe bleeding prior to intravenous administration of a small dose of radio-iron, the radio activity could be confined to a group of cells whose age was known within reasonable limits. As the cells matured and aged, measurements were made of their abilities to resist destruction by rotating glass beads and to resist hemolysis in hypotonic solutions of sodium chloride. Hemolysis of the total cell population was determined by conventional methods and hemolysis of the tagged cells by measurements of radioactivity. Comparison of the figures obtained revealed that cells up to 3–6 weeks of age were more resistant to mechanical trauma than the total cell population. The 2 fragility measurements then became almost identical until 12–15 weeks of age when the tagged cells were found to become increasingly fragile. The ratios then reversed after the tagged cells reached the end of their life span. A transient drop was noted in the total circulating radio-iron at this point corresponding to a life span of about 17 weeks for the tagged cells. The osmotic fragility of the tagged cells was considerably greater than that of the total cell population for the first week, confirming results obtained earlier by Cruz, Hahn, Bale, and Balfour (*Am. J. Med. Sci.*: 202, 157, 1941). Data for the older cells were inconclusive.

Parathormone and renal reabsorption of phosphate. HERBERT C. STOERK and ROBERT H. SILBER. *Merck Inst. for Therapeutic Research, Rahway, N. J.* Although it has been established that parathormone (PTH) activity is not dependent upon renal function, evidence has been presented both for and against an effect of PTH upon the reabsorption of phosphate by the renal tubules. In the present experiments, the renal excretion of phosphate during a 5-hour period was determined in parathyroidectomized and in intact rats after administration of graded amounts of phosphate (0, 4 and 8 mg. of P as Na_2HPO_4) and parathyroid extract (5–160 U, Lilly). In intact rats it was found that urinary phosphate excretion was not altered by the injection of 5 to 160 U of parathyroid extract. In parathyroidectomized rats the renal excretion of phosphate, but not of creatinine, was greatly suppressed. This defect was corrected by the administration of 20 U and no additional effect upon phosphate excretion was obtained with larger amounts of parathyroid extract (up to 160 U). In parathyroidectomized rats, injected with 20 or more U of extract, as well as in intact rats, an increased intake of phosphate led to an increase in urinary phosphate. It therefore appears that excessive amounts of parathyroid extract (more than 20 U per rat) exert no additional effect on the urinary excretion of phosphate. Thus the influence of PTH upon tubular reabsorption of phosphate is essentially an 'all or none' effect and can not be demonstrated in the presence of adequately functioning parathyroid tissue.

Eclampsia-like condition in pregnant rats injected with progesterone. A. SYMEONIDIS (introduced by H. L. STEWART). *National Cancer Institute, Bethesda, Md.* Pregnant rats (24, strain M-520) injected with 20 mg. of progesterone, intramuscularly (5 mg. every day) in late pregnancy became ill. Some of them aborted dead embryos following which they recovered. In other rats the embryos were absorbed. Approximately 40% of the treated animals died. The liver and the kidney showed striking gross and microscopic lesions resembling those of eclampsia. Elevation of the nonprotein nitrogen of the blood and albuminuria were found. The blood pressure was significantly elevated in 5 animals examined. Pregnant females receiving the same treatment prior to the 15th day of pregnancy and virgin tested rats failed to show any changes.

Relations between volumes of closed hypo-

thermal cerebral lesions and symptoms in rabbits. C. BRUCE TAYLOR (by invitation), GEORGE M. HASS and JOHN E. MALONEY (by invitation). *Rush Dept. of Pathology, Presbyterian Hospital, and Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago, Ill.* Acute closed cerebral lesions characterized by hemorrhage, necrosis and progressive edema were produced hypothermally in rabbits without interrupting continuity of the calvarium or introducing variables incidental to mechanical trauma. The dimensions and locations of lesions were controlled so that they could be reproduced in successive animals. Although hemorrhage, edema and necrosis varied slightly in lesions otherwise identical, the variations were restricted to discrete volumes of injury. When unilateral or bilateral lesions occupied less than 9.4 volumes % of the brain, symptoms were negligible. When lesions occupied 9.4 to 18.5 volumes % of the brain, severe symptoms developed in many animals. The minimum lethal volume of cerebral damage in 50 % of more than one hundred animals was 14.3% of the volume of the brain. Severe clinical courses with an average postoperative duration of 7 hours and fatal termination always occurred when lesions occupied more than 18.5 volumes % of the brain. Most animals that died had a normal postoperative period of behavior. Secondary lapse into stupor was a dependable indication of coma and eventual death within 24 hours after operation. These data indicate that a quantitative experimental approach to treatment of acute expanding closed intracerebral lesions characterized by local necrosis, hemorrhage and edema can now be made. The postoperative duration of life can be predicted from the magnitude of lesions produced and this duration is sufficient to permit evaluation of most therapeutic methods, now used empirically in treatment of similar lesions in humans.

Proteinuria in dogs. ROGER TERRY (introduced by G. H. WHIPPLE). *Dept. of Pathology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* If homologous plasma is given parenterally to normal dogs for a sufficient period, proteinuria develops. Additional experiments to those previously reported have been designed to help elucidate the mechanism of this proteinuria. Circulating plasma protein levels must be elevated above a critical 'threshold', and there appears to be a lag period between the development of hyperproteinemia and the onset of conspicuous proteinuria. This suggests that the 'threshold' for plasma protein molecules may be related to a large accumulation of these protein molecules within the renal tubule cells.

Actions of 4-amino-pteroylglutamic acid in dogs with special reference to hematopoiesis. J. B. THIERSCH and F. S. PHILIPS (introduced by C. P. RHOADS). *Division of Experimental Chemotherapy, Sloan-Kettering Inst. for Cancer Research, New York City.* Accepting 4-amino-pteroylglutamic acid as a folic acid antagonist from studies in rats (Philips and Thiersch, *J. Pharm. Exper. Therap., in press*) its actions in dogs were examined. Acutely toxic doses proved fatal within 3 to 4 days while daily doses (0.05 to 0.10 mg/kg.) were fatal to approximately 50% of animals within 10 days. Signs of intoxication included occasional emesis, anorexia, losses in weight and electrolyte, hemorrhagic diarrhea, leucopenia, hemoconcentration, and terminal collapse. In acutely intoxicated animals serial aspirations revealed rapid degeneration of bone marrow. Within 24 hours abnormal erythroid elements could be found including nuclear remnants, pathologic mitoses, and megaloblasts. After 96 hours erythroid elements were almost absent. Simultaneously, myeloid elements and megakaryocytes became decreased in number. Pathologic metamyelocytes and myelocytes appeared. In chronic intoxication, similar but less rapid degeneration of bone marrow occurred although megaloblasts were not found in all animals. In poisoned dogs lymphopoiesis was less affected, by comparison, than was either erythro- or myelopoiesis. Edema of intestinal mucosa and desquamating colitis with hemorrhage were present in all dogs.

Thermostable inhibition of bacterial hyaluronidases by serum of normal human beings. ROBERT T. THOMPSON and FRANCES E. MOSES (introduced by M. A. BLANKENHORN). *Dept. of Internal Medicine, College of Medicine, Univ. of Cincinnati, Cincinnati, Ohio.* This study investigated the inhibition of similar test strengths of hyaluronidases in culture filtrates of Type 3 Pneumococcus, hemolytic *Staphylococcus aureus*, *Clostridium perfringens*, beta hemolytic Streptococcus (group A), and purified bovine testicular hyaluronidase by the serum of 50 normal human beings who were between 20 and 40 years of age. Hyaluronidase inhibition was measured by a mucoprotein clot prevention test using egg albumin as the protein component of the substrate. Each serum was tested immediately as it was removed from the clot, and again after it had been heated at 56°C. for 30 minutes. Results tabulated below reveal thermostable inhibition of Pneumococcus and Staphylococcus hyaluronidases by normal serum:

Hyaluronidase tested	No. sera tested	Inhibiting sera	Thermostable inhibiting sera
Pneumococcus	50	47	45
Staphylococcus	49	23	19
Cl. Perfringens	50	16	10
Streptococcus	50	12	7
Testicular	48	46	2

Tests upon consecutive daily sera of 1 person revealed consistent inhibition of the pneumococ-

cus, staphylococcus, and testicular hyaluronidases. However, there was fluctuation in the inhibition of the Streptococcus and *Cl. perfringens* hyaluronidases, so that results of the inhibition of these 2 enzymes tabulated above are of undetermined significance. The inhibition of testicular hyaluronidase which disappeared after heating could be restored by the addition of complement to the heated serum. The complement used was a 1:30 dilution of normal guinea pig serum, which itself did not inhibit the test strength of hyaluronidase.

Association of lymphoid elements with cancer cells undergoing distinctive necrobiosis in resistant and immune hosts. HELENE W. TOOLAN (by invitation) and JOHN G. KIDD. *Dept. of Pathology, The New York Hospital—Cornell Medical Center, New York City.* Regression of C3H cancers in 'A' mice proceeds inwards from the periphery, the tumor cells in groups in the centermost parts of the regressing nodules, and often those surrounding blood vessels, continuing to undergo mitosis and showing no signs of injury until their more peripheral fellows are overcome. Scrutiny of the cells about the edges of the regressing tumors made it plain that they do not die 'en masse', but instead succumb one by one, manifesting as they do so a distinctive type of necrobiosis. This is characterized by a gradual but progressive shrinkage of the cell with intensified basophilism of its nuclear and cytoplasmic constituents, and often with the accumulation of fluid around it; eventually with further shrinkage the nuclear membrane breaks down and the condensed and darkly-staining cellular remains dwindle and disappear without the intervention of phagocytes. The tumor cells never exhibit the necrobiotic changes just described until lymphoid elements provided by the host accumulate in force about them and attach themselves to first one and then another of the outlying cancer cells, adhering closely to them and often curving like crescents about their rims as they shrink, while adjacent tumor cells without attached lymphocytes remain unaltered. Further studies showed that cancer cells of both types proliferate for a short time when implanted in 'A' mice in which they had previously grown and regressed, but with the accelerated arrival of lymphoid elements they are promptly overcome as described above.

Passive transfer of age resistance to an avian malaria parasite. WILLIAM TRAGER and R. BARCLAY MCGHEE (by invitation). *Dept. of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.* If young chicks are inoculated intravenously with the erythrocytic stages of *Plasmodium lophurae* they undergo an acute, severe infection. If adult chickens are similarly inoculated, few of them exhibit any appreciable parasitemia. It has now been found that the plasma of adult chickens contains a material which when injected into young chicks infected with *P. lophurae* is capable of reducing the extent of multiplication of the parasites. One-week old chicks were given a dosage of *P. lophurae* sufficient to produce an initial parasite count of 1 to 2 parasites per 100 red cells. They were then given an intravenous injection once daily for 2 to 4 days of 3 ml. of either heparinized adult chicken plasma, buffered saline (*pH* 7.4) or 6% bovine albumen in buffered saline. The chicks receiving adult chicken plasma consistently developed less severe infections than those receiving saline or bovine albumen. For examp e, in one experiment the averages of the peak parasite numbers were, as parasites per 100 red cells, 102 for the group receiving albumen, 47 for the group receiving hen plasma and 102 for the group receiving saline. The activity of the hen plasma was not destroyed by heating it for one half hour at 56°C.

Radioresistance and radiosensitivity of swine exposed to atomic bomb radiation. JOHN L. TULLIS (introduced by F. S. ROBSCHEIT-ROBBINS). *Commander, MC, U. S. Navy.* Irradiation of the total body with an atomic bomb source affords a fairly accurate method of studying the differences in radiosensitivity of tissues. The variables introduced by the body thickness of swine and the differences in specific density of the tissues are minimized by the use of atomic bomb radiation, but they are by no means eliminated. The most radiosensitive tissues in swine are the lymphoid elements, the bone marrow, the gonads and the intestinal epithelium. It is significant and interesting that the reticular cells (the stem cells) of the lymph nodules and bone marrow, the indifferent cells (the stem cells) of the immature testis, the primordial ova, macrophages and plasma cells are relatively radioresistant. That is to say, doses of total body irradiation from an atomic bomb, or other source of penetrating ionizing radiations, sufficient to kill swine often cause no morphologic alteration in the cells listed. If treatment during the acute phase of the injury is successful in maintaining life, there is histologic evidence that regeneration of radiosensitive cells can be accomplished by certain radioresistant apparently-uninjured stem cells.

Intradermal vaccination with infectious psittacosis virus: pathological changes. JOSEPH VICTOR and JOHN C. WAGNER (by invitation). *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.* Injection of psittacosis virus intramuscularly in monkeys (Rivers and Schwentker, *J. Exp. Med.* 60: 211, 1934) and intradermally in guinea pigs has produced resistance to respiratory challenge. Pathological effects of intradermal injection of guinea pigs of strain 6BC grown in chick embryo yolk sac (6BCYS) were studied at intervals during

5 weeks after injection. Distribution of virus in tissues of these animals is reported elsewhere. The infectious material produced acute inflammation at the injected site. The skin lesion later became granulomatous with fibroblast and giant cell formation after 3 weeks. Ceroid pigment occurred within histocytes and giant cells 3 weeks after injection of 6BCYS. Ceroid was identified from its fluorescence in U-V light, acid fastness with carbolfuchsin and its affinity for basic and lipophil dyes. Regional lymph nodes became hyperplastic and hypertrophied reaching maximum size at about 2 weeks. Ceroid appeared in these nodes at 5 weeks and seemed to have migrated from the skin lesion. Liver contained midzonal areas of focal necrosis for 2 weeks. After 3 weeks, collections of histocytes replaced the midzonal necrosis. Control animals injected intradermally with normal chick embryo yolk sac or formalinized yolk sac infected with 6BC revealed no changes comparable to those treated with infectious virus. Lesions in skin, regional lymph node and liver persisted longer than detectable virus. No virus was found in livers with focal necrosis.

Comparison of two types of experimental ascites in the dog. WADE VOLWILER (by invitation), JESSE L. BOLLMAN and JOHN H. GRINDLAY. *Mayo Foundation, Rochester, Minn.* Ascites in the dog has been regularly produced by *1*) chronic passive congestion of the liver from the application of a cellophane band to the thoracic inferior vena cava and 2) plasmapheresis following the application of a cellophane band to the abdominal inferior vena cava and portal vein. Although portal and inferior caval venous pressures are elevated to the same moderate degree in both types of preparations, the character of the ascites is markedly different. The ascites accompanying Preparation 1 is rapidly progressive, high in protein content, and regularly associated with marked engorgement of the extrahepatic liver lymphatics; whereas the abdominal fluid appearing in Preparation 2 is less extensive and difficult to maintain, low in protein content and without associated lymphatic enlargement. Evans blue dye injected intravenously into dogs of Preparation 1 is found in the ascites in one hour; the dye does not appear in the abdominal fluid of Preparation 2 in detectable amounts in 48 hours. Assays for antidiuretic substances in the urine concentrates of dogs of Preparation 1 have been made using male rats having suprapubic cystotomies for individual urine collections. Since previous studies have shown that the protein content of hepatic lymph is approximately $\frac{3}{4}$ that of plasma and that the rate of flow of liver lymph is markedly increased from hepatic congestion, it is suggested that increased transudation of fluid through the dilated extra-hepatic liver lymphatics contributes importantly to the formation of the ascites associated with hepatovenous congestion.

Intradermal vaccination with infectious psittacosis virus: virus distribution. JOHN C. WAGNER (by invitation) and JOSEPH VICTOR. *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.* Production of immunity to psittacosis virus introduced into respiratory tract has been reported in monkeys by Rivers and Schwentker, following intramuscular inoculation of infectious virus. Similar observations were made following intradermal administration of psittacosis virus, in guinea pigs. This study attempts to elucidate factors concerning this resistance. Tissues of guinea pigs injected with psittacosis virus were studied for distribution of virus and pathological changes. Psittacosis virus, 6BC strain, cultivated in chick embryo yolk sac, was inoculated intradermally in a concentration of 300,000 egg LD_{50} in 0.5 ml broth suspension. Autopsies were performed 1, 2, 3, 6, 9, 14, 21 and 35 days thereafter. Tissues were tested as 10% suspensions in nutrient broth by intracerebral inoculation of mice. Presence of virus in guinea pigs was established by demonstration of elementary bodies in smear preparations of brains from injected mice that died. Evidence obtained indicated that treated guinea pigs harbored virus for 6 days at site of inoculation, for 14 days in regional lymph nodes and sporadically for 9 days in the liver. Spleen suspensions yielded virus on the first day in only 1 of 3 animals. No virus was detected in suspensions of contralateral lymph nodes, whole blood, heart, lung or kidney. Virus distribution data indicated that psittacosis virus inoculated intradermally migrated from site of injection to regional lymphatics and thence to internal organs. Pathologic studies confirmed this. Resistance to respiratory infection occurred in the absence of any demonstrable virus or lung lesions.

Effect of renal ischemia on the production of nephrosis in jaundiced rabbits. W. B. WARTMAN, J. M. TUCKER (by invitation), A. P. RUSTERHOLZ (by invitation) and R. JENNINGS (by invitation). *Dept. of Pathology, Northwestern Univ. Medical School, Chicago, Ill.* The purpose of these experiments was to test the assumption that the presence of previous kidney damage might be a factor in the development of bile nephrosis. Using rabbits, one kidney was damaged by temporarily occluding the right renal artery and vein for 15 to 20 minutes, and the common bile duct was divided. Lesions developed in the kidneys of approximately 45% of animals after 3 days. They were chiefly in the cortex of the kidneys and consisted of dilatation of both proximal and distal segments of the tubules, flattening, degeneration and necrosis of the lining epithelium and numerous reddish brown, granular, heme casts. There were no clinical evidences of kidney disease. No changes were dis-

covered in rabbits in which only the common bile duct was cut, nor in animals in which only the right renal artery and vein were obstructed, nor did rabbits in which the common bile duct and right renal pedicle were dissected out but not otherwise manipulated show changes in the kidney. Prolonged and severe restriction of water intake had no demonstrable influence on the development of the nephrosis. It is concluded that under the conditions of these experiments, a peculiar type of bilateral nephrosis may be produced in rabbits by a combination of jaundice and temporary, unilateral, renal ischemia, and that it is produced neither by jaundice alone nor renal ischemia alone. Restriction of fluid had no effect upon the development of the lesion.

Bactericidal action of dog blood against Br. suis. E. BUIST WELLS (by invitation), JEAN CLEMENTS (by invitation), ABOU D. POLLACK (by invitation) and JOSEPH VICTOR. *Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.* Irwin et al. (*J. Inf. Dis.* 58: 15–22 1936) have reported that bovine plasma had greater bactericidal activity than whole blood against *Br. suis* and *Br. abortus*. The present study indicated that in the dog whole blood possessed great bactericidal activity against *Br. suis* whereas plasma had none. A standardized number of organisms of a virulent strain was mixed with either normal dog blood, red blood cells, buffy coat or plasma. Mixtures were agitated in rotating tubes. Bacterial numbers were counted by dilution and plating. The influence on bactericidal action of blood of citrate, heparin, temperature, time of mixing, volume of blood and interval between addition of fresh blood was studied. Citrate suppressed bactericidal activity of blood whereas heparin did not. Bactericidal activity of blood was the same both at room temperatures and at 37°C. after 2 hours. In contrast, after 4 hours bacterial destruction was greater at 37°C. than at room temperatures. Repeated additions up to 4 hours of fresh blood to the mixtures did not influence bactericidal action. Activity of buffy coat was similar to that of whole blood. Plasma was inactive. Blood from which almost all leukocytes had been removed by centrifugation possessed much less activity than whole blood. It appeared that bactericidal power of dog blood for *Br. suis* depended on the presence of leukocytes.

Clearance of rose bengal by livers of normal and carbon tetrachloride-treated mice. W. LANE WILLIAMS. *Dept. of Anatomy, Univ. of Minnesota, Minneapolis, Minn.* Livers of untreated mice were cleared of rose bengal (intraperitoneal, 0.4 cc., as a 1% saline solution 25 gm. body weight) within 4 to 6 hours post injection. Twenty-four hours after treatment with 0.4 cc. of CCl_4 (subcutaneously in mineral oil) total clearance time was 9 to 11 hours; at 48 hours, 11 to 14 hours. These livers showed typical centrilobular damage. Nine days subsequent to a single injection of 0.01 cc. of CCl_4, livers showed no histological evidence of parenchymal damage but dye clearance time was 8 to 9 hours. One day after injection of the larger amount of hydrocarbon all parenchymal cells (including peripheral ones) were more deeply stained (cytoplasm) than equivalent cells in livers of untreated animals. Greatest amount of dye was in centrilobular cells of injured livers. Although these central zones eventually showed necrosis the deeply stained centrilobular cells were still able to excrete dye 48 to 60 hours after injection of CCl_4. Intracytoplasmic rose bengal is difficult to determine in cytological studies because of its light pink color. However, the presence of a greater intracytoplasmic concentration of dye transiently 'labels' and differentiates damaged parenchymal cells. All such cells were free of dye within 8 to 14 hours after injection. In ultraviolet light 10μ paraffin sections of normal livers showed a faint yellow fluorescence during the period of greatest dye content. In CCl_4-damaged livers a definite orange fluorescence was observed during the interval of maximum dye content.

Vitamin deficiency and protein synthesis with special reference to riboflavin-protein interrelationships. ROBERT W. WISSLER (by invitation), EARL P. BENDITT, C. HAROLD STEFFEE (by invitation) and ROBERT L. WOOLRIDGE (by invitation). *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* The omission of all vitamins from the diet of normal young adult albino rats produced little change in weight gain or diet consumption for about 30 days. Under similar conditions protein-depleted rats showed poor diet consumption and weight loss in 2 or 3 days after being shifted to a low vitamin ration adequate in protein. When each vitamin was removed singly from protein-repletion rations, groups of protein-deficient rats displayed marked reduction in diet consumption and weight gain only when the diets were devoid of riboflavin or thiamin. Riboflavin deficiency usually began to become apparent in 3 or 4 days; thiamin in 7 to 10 days. Antibody production was markedly reduced in the riboflavin-deficient rats. Pair-feeding experiments revealed that the poor weight gains shown by the protein-depleted rats repleting on riboflavin-deficient diets were not the result of inanition alone. Analyses of the various tissue compartments revealed that the smaller weight gains of these riboflavin-deficient rats were the result of interference with protein synthesis as well as fat storage. Liver and carcass analyses indicated that riboflavin storage is decreased in the protein-depleted rats despite adequate riboflavin intake. Succinic dehydrogenase and other enzymic activity was reduced in the livers of protein-depleted animals and remained low in those repleting without

dietary riboflavin. Protein-depletion interferes with enzyme activity and riboflavin storage possibly by limiting available protein prosthetic groups.

Response of the rachitic metaphysis in the rat to excessive administration of vitamin A. S. B. WOLBACH and CHARLOTTE L. MADDOCK (by invitation). *Division of Nutritional Research, Children's Hospital, Boston, Mass.* It has been shown (*Proc. Inst. of Medicine of Chicago* 16: 1946; and *J. Bone and Joint Surg.* 29: 171, 1947) that in the growing young animal the administration of excessive amounts of vitamin A accelerates growth and maturation of epiphyseal cartilage cells and the remodelling sequences of the bones of the skeleton. This has been demonstrated for the rat, mouse, guinea pig and dog. It causes rapid consumption of the epiphyseal cartilage and, in appropriate species, premature closure of epiphyses, also excessive rapidity of remodelling processes in regions which undergo remodelling in normal growth. The present communication is for the purpose of describing effects upon the accumulated cartilage in rachitic metaphysis which results from failure of cartilage cells to mature. The rachitogenic diet employed was composed with the advice of Dr. Otto A. Bessey and contains all essential materials with the exception of adequate phosphorus content. Under the influence of excessive vitamin A administration there is rapid repair of rachitic lesions, including resumption of normal epiphyseal cartilage cell cytomorphosis and calcification of cartilage matrix and osteoid. Partial to complete closure of the distal, femoral and proximal tibial epiphyses takes place in spite of the fact that in these locations epiphyses remain open for most of the lifetime of the rat. Acceleration of remodelling sequences occurs as in rats upon normal diets and in conformity with normal growth patterns. Our results support the idea that epiphyseal cartilage cell sequences are influenced by a factor other than those concerned in calcification of matrices.

Effects of protein deficiency upon natural and acquired immunity to Friedlander's bacillus. ROBERT L. WOOLRIDGE (introduced by PAUL R. CANNON). *Dept. of Pathology, Univ. of Chicago, Chicago, Ill.* The effects of protein depletion on natural and acquired immunity against infection with Friedlander's bacillus were studied in adult male albino rats following intranasal or intratracheal introduction of a standardized inoculum. Forty-one markedly protein-depleted rats showed a significantly lower agglutinin response on the 6th and 14th days after antigenic stimulation, with a concomitantly lower survival rate, than did 40 well-nourished controls. When the animals were sacrificed the controls had only a few focal pulmonary abscesses whereas the protein-depleted rats had many large chronic pulmonary abscesses which showed evidence of progressive enlargement with approximately 50% of each lung consolidated. Marked protein depletion also lowered natural resistance in that an intratracheal inoculum which was sublethal for the control animals killed all of the protein-depleted rats within 54 hours, with an associated septicemia. Histological studies revealed marked differences in the cellular response and degree of phagocytosis. For example, in the protein-depleted rats the ability to circumscribe the infection and repair the damaged tissue was definitely impaired whereas the phagocytic ability of the control animals during the early stage of the infection was adequate to protect them until acquired immunity was developed and the infection localized.

AMERICAN INSTITUTE OF NUTRITION

Thirteenth Annual Meeting

DETROIT, MICHIGAN, April 18–22, 1949

(For possible corrections in any of the following abstracts see the June issue)

Leucine content and utilization of amino acid mixtures. JOSEPH T. ANDERSON (by invitation) and E. S. NASSET. *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* The utilization of the N of amino acid mixtures in the adult rat was investigated by the method previously described (Anderson and Nasset, J. Nutrition 36: 703, 1948) in which a 'complete' amino acid mixture simulating egg protein is compared with a mixture in which a portion of one of the essential amino acids has been replaced by an isonitrogenous amount of glutamic acid. Reduction of L-leucine to 21.1 mg. leucine N/gm. total N, which is ⅓ the value in the 'complete' amino acid mixture, caused a significant decrease in the utilization of the dietary N as indicated by a decrease in N balance index and an increase in N requirement for equilibrium. Further reduction of L-leucine by one-half caused a further decrease in N utilization but not so great as would be predicted from the theory that N retention is limited by the supply of limiting essential amino acid. Substitution of an equal quantity of DL-leucine for the L-leucine (21.1 mg. leucine N/gm. total N) resulted in no significant reduction in N utilization. Apparently about ½ the maintenance requirement of L-leucine in the rat can be filled by D-leucine.

Ammonium persulfate as a flour maturing agent. AARON ARNOLD and FRANS C. GOBLE (by invitation). *Sterling-Winthrop Research Inst., Rensselaer, N. Y.* The need for chemical agents for rapidly maturing flour is well established and accepted. Nitrogen trichloride, which has been used extensively for this purpose, is no longer acceptable in view of Mellanby's findings of deleterious effects in laboratory animals fed flour thus treated. This fact has focussed attention on the suitability of alternative agents. Ammonium persulfate has been known for some time to be an effective flour maturing agent, and, in fact, is used extensively abroad; this report presents data obtained in feeding trials of this compound with rats and dogs. In chronic experiments, rats were fed diets high in flour or air-dried bread made from persulfate treated flour. The levels of treatment were 0.02 and 0.1%, i.e., levels considerably in excess of commercial requirements. The rats fed the treated flours or breads showed no adverse effects. The effects were judged on the basis of growth and reproduction performance and histological examination of representative tissues of first and second generation rats sacrificed after 6 months on test. Dogs fed ammonium persulfate treated flour not only did not develop the running fits syndrome but were cured of running fits when transferred from a diet containing nitrogen trichloride treated flour to one containing persulfate treated flour. As in the case of the rat feeding trials, no adverse effects could be detected in dogs fed flour treated with 0.15% persulfate as compared with dogs fed untreated flour. The effects were judged from weight records, kidney and liver function tests, and histological examination of representative tissues after 5 months' feeding. Ammonium persulfate appears to be suitable from all view points for use as a flour maturing agent.

Effect of diet on treatment of parathyroidectomized dogs. AARON ARNOLD. *Sterling-Winthrop Research Inst., Rensselaer, N. Y.* It has been well demonstrated that vitamin D and dihydrotachysterol will substitute for parathyroid hormone in parathyroidectomized dogs. However there is a disagreement as to the amounts of the calcemic steroids required. For 16 months, 2 adult thyroparathyroidectomized dogs were fed a mixture of 2 parts fresh ground meat and 1 part cereal diet (in percentage: corn 26, wheat 26, skim milk powder 24, linseed meal 8, casein 4, peanut oil 4, yeast 3, alfalfa meal 3, bone meal 1, $CaCO_3$ 0.5, and NaCl 0.5). The composite diet supplied 0.58% Ca and 0.73% P. The dogs were given, in addition, 0.3 gm. thyroid powder daily and the usual vitamin supplements orally once weekly. In agreement with the results of McChesney and Giacomino (J. Clin. Invest. 24: 680, 1945), the dogs were observed to require approximately 0.0875 mg. of dihydrotachysterol or 0.2 mg. of calciferol daily per 10 kg. dog. This amount of calciferol was about 200-fold greater than that found necessary by Kozelka *et al.* (J. Biol. Chem. 100: 715, 1933). The requirement does not appear to be correlated with the dietary calcium and phosphorus but may be related to the general nature of the diet. Specifically, dogs fed a diet with a substantial proportion of fresh meat require much greater quantities of vitamin D or dihydrotachysterol than do dogs fed diets com-

posed largely of cereal grains. This suggestion may apply also to the marked difference in the amount of vitamin D required to prevent the onset of rickets in dogs as reported by the Fleischmann Laboratories (unpublished data, 1936) and by Arnold and Elvehjem (J. Am. Vet. Med. Assoc. 95: 187, 1939).

Influence of heredity on the crude protein and carotene contents of corn. L. W. AURAND (by invitation) and R. C. MILLER. *Dept. of Agricultural and Biological Chemistry, Pennsylvania State College, Penna.* Crude protein and carotene values have been determined on corn produced in 3 different series of experiments, each of which had all possible single cross combinations of 10 different inbred lines of corn. Each experiment was completely replicated on at least 3 experimental plots which had the same fertilizer treatment. Separate samples of corn from each single cross combination on each plot were analyzed for protein and carotene, and average values were computed from all of the individual data for each single cross on each experiment. Significant differences in the protein and carotene values for single crosses definitely reflected the influence of the breeding of the inbred lines on the resulting single crosses. The latter had crude protein contents ranging from 7.95 to 12.58% and carotene contents ranging from 0.53 to 1.95 mg/lb., all values being expressed on the basis of 15.5% moisture content of the corn. The definite influence of inbred lines was shown in characteristically high or low crude protein and carotene contents of the resulting single cross combinations. Although these relationships were true for both crude protein and for carotene there was no correlation between the crude protein and carotene contents of corn tested. The results show a definite influence of heredity on the carotene and crude protein content of corn and point the way for improvement of the nutritive value of corn by the plant breeder.

Nutritional requirements of men living under survival conditions in cold weather. C. G. BLY (by invitation), R. E. JOHNSON and C. F. CONSOLAZIO (by invitation). *U. S. Army Medical Nutrition Laby., Chicago, Ill.* Balance studies were conducted when 32 volunteers, fully acclimatized to Florida winter conditions and unaccustomed to cold weather and its hardships were flown in January from Florida (mean temperature +57°F.) without layover to Manitoba, Canada. On arrival they were placed in a field survival situation for 12 days (mean temperature −3°F.), where they lived in one of four segregated areas, 8 men per group. The 4 group rations (per man per day) contained: 1100 Cal. (low calorie control); 1650 Cal.; 1940 Cal.; 4850 Cal. (high calorie control). All urine and feces were collected daily. From analytical data on these, together with analytical data on all foods and fluids consumed, nutrient balances were computed. For these young men, well outfitted against the cold, with adequate clothing, fuel, and tentage, doing no physical work other than necessary to keep themselves comfortable, nutrient balance could be maintained for 12 days by palatable rations supplying daily for an average 70 kg. man: 3400 Calories; 2.7 liters water, including beverage, food, and metabolic water; 50 gm. protein, mostly animal; 135 gm. fat; 400 gm. carbohydrate; 1.1 gm. calcium; 1 gm. phosphorus; 100 mEq. sodium; 85 mEq. chloride; 20 mEq. potassium. These estimates are not applicable for men required to perform any hard physical work.

Animal protein factor and growth of mice fed diets containing succinylsulfathiazole. D. K. BOSSHARDT, J. W. HUFF (by invitation), WINIFRED J. PAUL (by invitation) and R. H. BARNES. *Dept. of Biochemistry, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Penna.* The addition of 2% succinylsulfathiazole to purified diets fed to mice caused a growth retardation that was not corrected by the feeding of biotin and pteroylglutamic acid nor by supplements of dehydrated, defatted whole liver. When, however, the biotin, pteroylglutamic acid, and liver were fed together growth rates were obtained comparable with those of mice fed a succinylsulfathiazole-free diet containing liver. Liver has been shown to contain a factor, or factors, referred to as the animal protein factor that is necessary for growth. The results of Ott *et al.* (J. Biol. Chem. 174: 1047, 1948) suggest that vitamin B_{12} would replace liver in the diet of succinylsulfathiazole-fed mice. The vitamin B_{12} concentrate was prepared from liver and was found by microbiological assay, using *Lactobacillus leichmannii*, to contain approximately 1% vitamin B_{12}. A vitamin B_{12} concentrate supplied by the Merck Laboratories was used as a standard. This preparation when administered to iodinated casein-fed mice (Bosshardt *et al.* J. Nut., in press) gave a maximum growth response with a daily intraperitoneal dosage of 0.5 μg. The vitamin B_{12} concentrate administered by intraperitoneal injection at a level of 1 μg/day to mice fed a diet containing biotin, pteroylglutamic acid, and succinylsulfathiazole produced a marked growth response. However, the growth response did not appear to be as great as that obtained with crude liver.

Riboflavin and nitrogen metabolism of 6 women with active tuberculosis. WILMA D. BREWER (by invitation), HELEN L. TOBEY (by invitation) and MARGARET A. OHLSON. *Dept. of Foods and Nutrition, School of Home Economics, Michigan State College, East Lansing, Mich.* The riboflavin and nitrogen metabolism of 6 women with moderately advanced, active tuberculosis was studied. The subjects were non-ambulatory patients of the county sanatorium and the regular hospital diet

was served throughout the experiment. The riboflavin and nitrogen contents of the diets were varied by adjustment of the milk intake. During periods 1 and 2, the milk intake was restricted to 300 gm. daily and for periods 3 and 4, the intake was increased to 1200 gm. of milk per day. Daily supplements of 5 mg. riboflavin were given during periods 2 and 4. Fourteen days of dietary adjustment preceded each 7-day balance period during which the intakes and excretions of nitrogen and riboflavin were determined. The urinary excretion of riboflavin following a test dose of 3 mg. riboflavin was measured on the last day of each balance period. The average daily intakes of riboflavin for the four periods were 1.35, 6.41, 2.60 and 7.79 mg. The average daily excretions were 0.31, 3.53, 1.08 and 5.63 mg. riboflavin, respectively. The retentions of nitrogen by the tuberculous women were similar to those of healthy women at comparable intakes. All of the subjects were in nitrogen equilibrium or retaining nitrogen at intakes above 10.00 gm. daily. Analysis of covariance has been applied to the data and the possible interrelationships of riboflavin and nitrogen metabolism will be discussed.

Lysine requirement for growing pigs. M. J. BRINEGAR (by invitation), J. K. LOOSLI, HAROLD H. WILLIAMS, and L. A. MAYNARD. *Depts. of Animal Husbandry and Biochemistry and Nutrition, Cornell University, Ithaca, N. Y.* Various levels of l-lysine monohydrochloride were added to a diet containing linseed meal supplemented with histidine and methionine for pigs averaging 19 kg. in weight. The basal diet consisting of 10.6% protein contained 0.34% lysine as determined microbiologically using Leuconostoc mesenteroides. The experimental diets were made up to contain 0.34%, 0.42%, 0.50%, 0.58%, and 0.74% pure l-lysine. The first 4 levels of lysine were fed to 3 pigs each and the 0.74% level was fed to 2 pigs. Protein efficiency ratios over a 4-week feeding period and biological values during a 5-day nitrogen balance were studied. The averages of the gm. gain in weight per gm. protein consumed were 2.6, 2.85, 3.12, 3.47, and 3.49 for each of the respective lysine levels of 0.34%, 0.42%, 0.50%, 0.58%, and 0.74%. The average biological values for the corresponding lysine levels were 52, 51, 61, 73, and 72. Each level of dietary lysine up to 0.58% increased the protein efficiency and the biological value, but the 0.74% level caused no further improvement. These data show that with a diet containing 10.6% protein, having a biological value of 73, growing pigs require approximately 0.6% of l-lysine in the ration.

Acetylation in diabetic rats. FRIXOS CHARALAMPOUS (by invitation) and D. MARK HEGSTED. *Dept. of Nutrition, Harvard School of Public Health, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* The ability of alloxan-diabetic rats to acetylated p-aminobenzoic acid (PAB) was compared to that of normal rats by injecting 1 mg. of PAB intraperitoneally, collecting the following 24-hour urine, and analyzing the urine for total and free PAB. All animals received a purified diet containing casein, salts, glucose, corn oil, cod liver oil and riboflavin, thiamine, nicotinic acid, pantothenic acid, and pyridoxine. Acetylation gradually decreased after the onset of diabetes and eventually fell to 62% of the dose as compared to 76% in the non-diabetic controls. Induced diuresis in the normals did not decrease acetylation. Acetylation in the diabetic animals returned to normal after the injection of insulin, citrate, α-ketoglutarate, succinate, fumarate, malate, oxaloacetate, diacetyl, acetylphosphate, and ATP. Increasing the level of fat in the diet from 5 to 30% had a similar effect, as did starvation for 24 hours. Injections of pyruvate, lactate, glyceryl diacetate, acetoin, butylene glycol, methyl ester of acetoacetic acid, phosphate salts, adenylic acid, and pantothenic acid failed to restore acetylation to normal in the diabetic rats. None of the compounds tested affected the acetylation in normal animals. These findings are in agreement with the *in vitro* work of Lipmann *et al.* (J. Biol. Chem., 160, 173, 1945) that acetylation requires high energy phosphate compounds. It appears that in the diabetic animal the regeneration of such high energy compounds is below normal.

Excretion of ascorbic acid on supplements of cantaloupe and crystalline ascorbic acid. SHIH DZUNG CHEN (by invitation) and CECILIA SCHUCK. *Nutrition Laby., School of Home Economics, Purdue Univ., Lafayette, Ind.* Four senior college women majoring in nutrition served as subjects. The study was divided into 3 periods: a saturation period of 5 days during which time the subjects, while consuming their regular diets, were given 200 mg. of ascorbic acid daily in tablet form; a test period of 6 days on a constant basal diet supplemented daily with 40 mg. of pure crystalline ascorbic acid; a second test period with approximately the same ascorbic acid intake, but with cantaloupe as the supplement to the basal diet. The 2,4-dinitrophenylhydrazine method for reduced and dehydro ascorbic acid (J. Biol. Chem. 174: 201, 1948) was used for the analysis of the diet and the cantaloupe supplement and for determining the urinary excretion. Since the excretions for the first 2 days of the 6-day test periods reflected the large intake of ascorbic acid during the saturation period these were not considered in the evaluation of the data. With an intake of 74 mg. of ascorbic acid (reduced and dehydro) the average daily amounts excreted during the crystalline ascorbic acid period by the 4 subjects were 49.24, 47.65, 47.43, and 38.30 mg. respectively. Corresponding amounts excreted during the cantaloupe period with a daily intake of 72 mg. ascorbic acid

were 57.54, 58.63, 41.88, and 34.81 mg. The figures show an increased excretion during the cantaloupe period for 2 of the subjects and a slightly decreased excretion for the other two.

Determination of SLR factor in the liver and urine of rats fed on various diets. Bacon F. Chow, Lois Hall (by invitation), Shirley De Biase (by invitation) and Lois Barrows (by invitation). *Division of Pharmacology, The Squibb Inst. for Medical Research, New Brunsweick, N. J.* Protein depletion causes a marked decrease in protein and enzyme contents of the liver. This report deals with the effect of inanition on the concentration of the SLR factor (Elvehjem, J. Biol. Chem. 167 : 86, 1947) in the liver of rats. It was found that if the liver were homogenized with an ice cold saline solution immediately after its removal from the animal, the activity of such a suspension increased some 10-fold upon incubation at 37°C. A maximum was, however, reached after 8–24 hours at this temperature. The results of assay of this factor in the homogenized livers of normal and seven-days-fasted rats, after appropriate length of incubation, demonstrated that inanition brought about a marked loss in the total amount of the potential activity, when expressed in units of activity per hundred grams body weight of the animals before fasting. The excretion of the SLR factor in urine was also studied. The renal excretion of this factor decreases on feeding a diet low in protein but adequate in calories and vitamins, but increases, if liver is given as a supplement either orally or intraperitoneally.

Weight of liver and liver glycogen of rats after dietary restriction and rehabilitation. Ruth M. Clayton and Esther da Costa (introduced by Robert E. Johnson). *U. S. Army Medical Nutrition Laby., Chicago, Ill.* Liver weights of 72 male rats fed during 13 weeks either a low calorie, modified or synthetic vegetable diet and of 120 rats changed from these diets to either high protein, high fat or high carbohydrate were determined. The glycogen of the liver of half of these rats was determined. The average weight of 42 control livers was 12.16 gm. with a total glycogen of 0.24 gm. The average weights of livers following the low calorie, modified carrot or synthetic vegetable diet were 7.13, 7.5 and 5.47 gm., respectively. The average total liver glycogens following the low calorie, modified carrot or synthetic vegetable diet were 0.12, 0.24 and 0.06 gm., respectively. After 72 hours of rehabilitation with a high protein, high fat or high carbohydrate diet following the low calorie diet were 13.2, 10.19 and 10.98 gm., respectively. Average liver weights from rats refed following the modified carrot or synthetic vegetable diet followed a similar pattern of increased weight. After 72 hours of rehabilitation liver glycogen increased in all diets but decreased after 96 hours. After 13 weeks of rehabilitation on a highprotein diet, liver glycogens were at the control level, on a high fat diet below and on a high carbohydrate diet above the control level.

Function of the ceca in the domestic fowl. J. R. Couch (by invitation), D. R. Knight (by invitation), Harry German (by invitation) and P. B. Pearson. *Agricultural and Mechanical College of Texas, College Station, Texas.* Broad-Breasted Bronze mature turkey toms and White Leghorn pullets were each selected at random from flocks on range and killed. The gastrointestinal tracts were removed and separated into two parts for sampling. The first section was made up of the ceca while the second consisted of the tract below the duodenum less the ceca. The contents were removed from each of these sections and analyzed for riboflavin, niacin, pantothenic acid, folic acid and biotin. The cecal contents contained approximately 1.5 times as much niacin, twice as much riboflavin, 2.5 times as much pantothenic acid, 6 to 8 times as much folic acid, and 10 times as much biotin as the intestinal contents. Identification studies of the microflora of the two sections of the GI tract showed that essentially the same flora existed in the ceca as in the rest of the tract. The ceca was removed from pullets which were placed in individual cages with raised screen bottoms. After 2 months a 24-hour collection of feces was made from the cecectomized birds and from normal pullets. There was no difference in the riboflavin, niacin, pantothenic acid, folic acid and biotin content of feces of cecectomized and normal birds fed a practical all-mash diet. These birds were then placed on a low biotin diet with starch as the carbohydrate. Biotin content and hatchability of the eggs will be reported as measures of intestinal synthesis in birds with and without the ceca.

Desoxypyridoxine and development of the chick embryo. W. W. Cravens and E. E. Snell. *Depts. of Poultry Husbandry and Biochemistry, Univ. of Wisconsin, Madison, Wis.* Injecting 1000 μg. of desoxypyridoxine into the hen's egg at 0 hours of incubation results in nearly 100% mortality of the embryos by the end of the fourth day of incubation and all embryos died prior to hatching; 250 and 500 μg. injected at 0 hours caused a reduction in hatchability, but some live chicks were obtained. These inhibitory effects were prevented by simultaneous injection of sufficient amounts of pyridoxal·HCl, pyridoxamine·2HCl or pyridoxine·HCl. The ratio of vitamin to inhibitor which permitted approximately 50% of the embroys to produce live chicks were: pyridoxal·HCl 1/20; pyridoxamine·2HCl 1/50; and pyridoxine·HCl 1/100. In contrast to the results at 0 days, injection of 100 μg. of desoxypyridoxine at 6 days of incubation was innocuous. At this time the toxic level was found to be 2.5–5.0 mg/egg: However,

this toxicity was not due to a competitive antivitamin action of the inhibitor since it could not be prevented by any of the three forms of vitamin B_6. At similarly high levels (more than 2.5 mg/egg) pyridoxal·HCl proved toxic. Pyridoxine·HCl was somewhat less toxic while pyridoxamine·2HCl was non-toxic at the highest level tested (10 mg/egg).

Effect of dietary restriction and rehabilitation on serum protein of male rats. ESTHER DA COSTA and RUTH CLAYTON (introduced by ROBERT E. JOHNSON). *U. S. Army Medical Nutrition Laby., Chicago, 9, Ill.* The mean total serum protein of 42 control rats was 6.4 gm.% and the A/G ratio was 1.34. Seventy-two rats kept 3 weeks on a low calorie, modified carrot or synthetic vegetable diet had total serum protein values of 6.14, 5.41 and 6.21 gm.% and A/G ratios of 1.43, 1.16 and 1.05 respectively. After 13 weeks of rehabilitation following the 3 types of restriction 120 rats were rehabilitated with a high protein, high fat or high carbohydrate diet. After 48 and 96 hours of refeeding, the high protein and high fat rats had slightly higher serum proteins than the controls but the high carbohydrate rats had significantly lower serum proteins. After 13 weeks of rehabilitation all types of refed rats showed normal protein values. During the first 48 hours of rehabilitation the A/G ratio of the rats on the high protein and high fat diets fell below 1.0, i.e. due to a lowering of the albumen. By 96 hours of refeeding all A/G ratios had reached 1.0 and by 6 weeks of refeeding they returned to normal. The rats kept on a low calorie, modified carrot or synthetic vegetable diet after 13 weeks lost an average of 39.6, 42.2 and 44.5% in weight but only the rats on the modified carrot diet had serum protein concentrations below normal.

Tryptophan—nicotinic acid relationship in the developing chick embryo. CHARLES A. DENTON (by invitation) W. L. KELLOGG (by invitation), W. E. ROWLAND (by invitation) and H. R. BIRD. *Bureau of Animal Industry, Agr. Res. Adm. U. S. D. A., Beltsville, Md.* The developing chick embryo synthesizes nicotinic acid and at the same time decreases in tryptophan content. The eggs contained on the average 36 micrograms of nicotinic acid, whereas the average content of the chicks was 1080 micrograms. The tryptophan decreased from 116 mgs. (average content of the eggs) to 72 mg. (average content of the chicks). Injection of 25 mg. of tryptophan into the eggs on the 10th day of incubation resulted in a significant increase in the nicotinic acid content of the chicks. Injection of 10 mg. of nicotinic acid into the eggs caused a slight increase in the nicotinic acid content of the chicks, but did not change the tryptophan content.

Effects of pteroylglutamic acid, injectable liver extract, and ascorbic acid on dietary glycine toxicity. JAMES S. DINNING (by invitation), CECILIA K. KEITH (by invitation), JOHN R. TOTTER and PAUL L. DAY. *Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock, Ark.* A study was made of the effect of pteroylglutamic acid (PGA), injectable liver extract (LE), and ascorbic acid on growth and fecal porphyrin excretion of rats receiving purified diet containing 10% glycine. The purified diet without glycine was fed to a control group. The average weekly gains over a 5-week period for the groups were: control 25.3 gm., high glycine 5.6 gm., high glycine + PGA 19.8 gm., high glycine + LE 12.9 gm., high glycine + PGA + LE 21.1 gm., and high glycine + ascorbic acid 9.3 gm. Both PGA and LE significantly improved growth when added to the high glycine diet ($p < 0.01$). PGA was more effective than LE in improving growth under these conditions and a combination of PGA and LE did not improve growth above the level obtained by PGA alone. Although addition of ascorbic acid to the high glycine diet improved growth slightly, the increase was not statistically significant. The fecal porphyrin excretion of rats receiving the high glycine diet was considerably lower than that of the control rats. Additions of PGA, LE, or ascorbic acid to the high glycine diet increased the fecal porphyrin excretion. The greatest increase in porphyrin excretion was obtained by the addition of a combination of PGA and LE to the high glycine diet; fecal porphyrin excretion under these conditions was elevated above that of the control rats.

Vitamin B_{12}—a growth factor for young rats. GLADYS A. EMERSON, ELIZABETH WURTZ (by invitation) and MARY E. ZANETTI (by invitation). *Merck Inst. for Therapeutic Research, Rahway, N. J.* The rat requires a factor which is present in liver and other animal proteins for normal growth and successful propagation. The influence of vitamin B_{12} on the progeny of rats maintained during gestation and lactation on a diet containing 60% soybean meal has been investigated. One group of animals received 5γ of vitamin B_{12} daily from impregnation while a like number of untreated females served as controls. The size and birth weights of the litters cast by the rats in each group were the same. However, the weaning weights (28 days) of the young from mothers receiving vitamin B_{12} averaged 50% more than did those from the untreated females. The offspring from each group were segregated at weaning into 2 subdivisions; in one the animals were given 0.5γ of vitamin B_{12} daily for 90 days and the others were unsupplemented. The weight increments of the males from the several groups were as follows: control mothers, young undosed, 151 gm.; control mothers, young dosed, 257 gm.; treated mothers, young undosed, 229 gm.; treated mothers, young dosed, 283 gm. The feeding of vitamin B_{12} to the young of control mothers resulted in body weights that were 100 gm. in excess of those of the unsupplemented animals.

Since the undosed young of the treated mothers grew for 2 months at a rate approximating that of the dosed young from the control mothers, it would appear that vitamin B_{12} is stored during the suckling period. The red and white cell counts of the individuals of all groups fell within the normal range.

Relation of choline intake to survival in rats fed p-dimethylaminoazobenzene. R. W. ENGEL. *Lab. of Animal Nutrition, Alabama Polytechnic Inst., Auburn, Ala.* When immature male and female rats of approximately 200 gm. body weight were subsisted for 2 months on a low-protein diet deficient in choline, they gained an average of 1.2 gm/rat/week. Inclusion in the diet of p-dimethylaminoazobenzene at the .06% level for a subsequent 7-week feeding period resulted in the death of 7 of 8 rats with an average survival period of 28 days. When the diet was fortified with choline (2 gm/kg.), the average body weight gain during the initial 2-month feeding period was 3.0 gm/rat/week and 8 of 8 rats survived the subsequent 7-week feeding period when p-dimethylaminoazobenzene was included in the diet at the .06% level. All the animals receiving the choline deficient diet exhibited gross evidence of liver cirrhosis and a marked accumulation of a clear serous fluid in the body cavities at autopsy. Only mild kidney damage was evident. The livers of the control animals receiving choline were normal in appearance, and no gross pathologic changes were evident in any tissues at autopsy after 7 weeks of p-dimethylaminoazobenzene feeding. The basal diet employed consisted of alcohol-extracted casein 2, alcohol-extracted peanut meal 10, sucrose 63.5, salts 4.4, L-cystine 0.1, lard 19, and cod liver oil 1, and was fortified with known accessory factors.

Chronic vitamin A deficiency in 3 inbred strains of mice. PAUL F. FENTON (by invitation), RUTH E. SHRADER (by invitation) and GEORGE R. COWGILL. *Yale Nutrition Laby., Dept. of Physiological Chemistry, and Dept. of Anatomy, Yale Univ., New Haven, Conn.* Weanling mice of the C_{57}, A and I strains were fed an artificial diet containing no added vitamin A. Growth rates of the C_{57} mice were less than those of the A strain although substantial growth occurred in both strains. Growth was more seriously retarded when the corn oil of the diet was replaced by hydrogenated cottonseed oil and vitamin E was omitted. A striking early feature of vitamin A deficiency in C_{57} mice was the graying or bronzing of the normally black fur. Distribution of the fur changes differed markedly from that seen in pantothenate deficiency. The vitamin A content of the livers was reduced to a very low level. Most mice survived many months without outward symptoms of deficiency. A substantial group was sacrificed when about 9 months old and organ weights determined. The most consistent findings regarding the blood picture were reductions in the red cell counts, hemoglobin levels and hematocrit values. Infection was frequently present. Many animals retained extensive fat depots despite 8 months on the deficient diet. Histological and histochemical studies were carried out on liver, kidney, spleen, thymus, testes and adrenals, and, when necessary, on other tissues. Several vitamin A-deficient male mice were injected with three doses of estradiol benzoate. Two months later the accessory reproductive structures were found to be greatly distended. They presented a histologic picture similar to that seen in mice treated for a long time with large doses of the estrogen.

Unidentified vitamins of the B complex required by certain insects. G. FRAENKEL (introduced by H. E. CARTER). *Dept. of Entomology, Univ. of Illinois, Urbana, Ill.* Many insects which normally live on grain or flour can be easily grown on 'synthetic' diets and are excellent material for nutritional studies. It is now an established fact that insects in general require all the known vitamins of the B-complex, including biotin and pteroylglutamic acid, with the exception of paraaminobenzoic acid and possibly inositol. Some insects seem to develop optimally on 'synthetic' diets in the absence of any sources of unidentified factors. With the mealworm, *Tenebrio molitor*, there is clear-cut evidence for the need of one additional factor present in yeast or liver, tentatively called B_T, which is not adsorbed on charcoal and has proved very elusive in all attempts to be precipitated from the aqueous phase. In this respect, and certain chemical characteristics as well, it bears a close resemblance to strepogenin, though the absence of B_T activity in casein excludes identity of the two factors. The need for at least 2 other unidentified members of the B-complex has been demonstrated for the flour beetle, *Tribolium confusum*. Growth on a 'synthetic' diet is slow by comparison with an optimal natural diet—wholemeal flour. A certain improvement is achieved by adding 2½% of the water-insoluble fraction of yeast, but a still greater improvement results from adding 1% dry yeast. For reasons still unexplained these deficiencies are more marked if starch is used as the carbohydrate, as compared with glucose. B_T concentrates failed to have any effect on *Tribolium* in diets deficient in one or two of the missing factors.

Effect of gossypol on the body weight of rats. ALFRED H. FREE and HELEN M. FREE (by invitation). *Miles Research Labys., Elkhart, Ind.* Gossypol is an organic compound present in the pigment gland of the cottonseed. It has long been recognized that the inclusion of large amounts of unheated cottonseed meal in the diets of animals has a detrimental and toxic effect which has been ascribed to gossypol. Zucker and Zucker (Abstracts of Papers, 111th meeting, American Chemical So-

ciety, page 47B, 1947) have suggested that gossypol has a specific effect in reducing food intake. Further experiments have been carried out in this laboratory to determine the effect of gossypol on the body weight of rats. Gossypol was prepared from whole cottonseeds by the method of Boatner et al. (Jour. Am. Chem. Soc. 69: 1268, 1947). Growth studies with young rats were made in which the animals were maintained on a standard diet and were given daily supplements of gossypol suspensions by stomach tube. In 7 experiments of this type employing 3 different preparations of gossypol, uniform results were obtained in which animals on higher intakes of gossypol died, whereas those on intakes that were ½ or ¼ of the toxic amount grew at rates comparable to the control group which did not receive gossypol. Adult rats showed no significant weight change when given a daily supplement of gossypol that was one-half the toxic amount. Starvation experiments with rats indicated that the weight loss of animals receiving minimal lethal quantities of gossypol was much less than the weight loss of animals dying of starvation.

Rat-repletion studies with amino acid solutions. Rôle of non-essential amino acids. DOUGLAS V. FROST and HARRY R. SANDY (by invitation). *Nutritional Research Division, Abbott Labys., North Chicago, Ill.* Weight response of adult protein-depleted rats to dry proteins, liquid protein hydrolysates or solutions of amino acids fed separately from protein-free diet was studied at controlled critical levels of nitrogen. A 5% solution was fed to supply twice Cannon's essential amino acid minima of tryptophan 13, phenylalanine 37, leucine 77, isoleucine 66, methionine 35, threonine 45, lysine 51, histidine 22 and valine 53 mg/rat/day. Discounting the D-isomers of isoleucine, threonine and valine, this provided about 100 mg. N/day. Only half of the liquid allotment representing this mixture was consumed and the weight response was low. Intake and response were nearly doubled by isonitrogenous replacement of 10 parts of essentials by arginine or glycine. Nitrogen efficiently ratios were high in each instance and compare favorably with the highest values calculated for standard proteins. Glutamic acid, in presence of arginine, glycine and alanine, led to a further improvement in acceptability and weight response. Complete hydrolysates of fibrin fortified with tryptophan were taken with greater avidity than amino acid mixtures, even though nitrogen efficiency was somewhat lower for the former. These effects were checked by repeated experiments in paired groups. The data suggest that absence of nitrogen other than essential amino acid N imposes a physiologic burden which is reflected in decreased appetite, but not in decreased nitrogen efficiency. Possible inhibitory effects of the D-amino acids may account in part for the lesser avidity for amino acid mixtures than for complete protein hydrolysates.

Decrease in the storage of thiamine in tissues from supplementing the diet with live bakers' yeast. FUNG-HAAN FUNG (by invitation), RUTH J. AANESS (by invitation) and HELEN T. PARSONS. *Dept. of Home Economics, Univ. of Wisconsin, Madison, Wis.* It has been established that when live bakers' yeast is ingested by human subjects, not only the yeast thiamine but also food thiamine consumed simultaneously fails to be returned in the urine in normal amounts, presumably because it is taken up in the digestive tract by yeast cells. However, about 80% of the nitrogen of the yeast cells was returned in the urine of the subjects. The hypothesis of an anti-enzyme analogous to folic acid anti-conjugase was untenable inasmuch as free thiamine was itself influenced by live yeast. Hence it became important to establish by direct proof whether or not absorption and storage of thiamine were actually interferred with by live yeast. Weanling pigs were found to be satisfactory test animals. Those fed live bakers' yeast in addition to the basal ration stored only about ½ as high a percentage of the total thiamine intake as pigs without this supplement. In all animals tested, about $\frac{9}{10}$ of the total storage occurred in the skeletal muscles. Supplementing with live yeast depressed the urinary thiamine and increased the fecal values quite comparably with previous results in this department on college women. This fact, considered with the decrease in thiamine storage in the tissues of pigs fed the yeast dose, substantiates the earlier conclusion that the ingestion of live bakers' yeast interferes with the absorption of thiamine by human subjects.

Biotin deficiency in ducklings and the histopathology of the heart. JAMES W. GODDARD and ROBERT E. OLSON (introduced by FREDERICK J. STARE). *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.* Studies in biotin-deficient ducks (J. Biol. Chem. 175: 503, 1948) have shown that the respiration, pyruvate and succinate utilization of cardiac muscle slices *in vitro* is markedly depressed below normal. In view of this the histopathology of the heart in biotin deficiency was investigated. Biotin deficiency was induced in one-day old ducklings by feeding a purified diet low in biotin and supplemented with 20% egg white. Deficient ducks were killed by decapitation at intervals after their growth had ceased and perosis developed, the hearts removed, weighed, fixed *in toto* in Romeis' fixing fluid and paraffin sections stained with Masson's trichrome, Mallory's phosphotungstic acid hematoxylin, and Heidenhain's iron hematoxylin. Grossly, the hearts from the deficient ducks were larger, heavier (50%) and paler than those from pair-fed controls. Microscopically, the hearts from the deficient ducks showed disruption

and lysis of myocardial fibrils, disintegration of anisotropic bands, and granularity and vacuolation of the muscle fibers. The Purkinje cells showed marked disorientation and disappearance of their myofibrils. In severe cases, these myofibrils and even whole Purkinje cells were replaced by connective tissue. The endocardial cells showed occasional hyperplasia, chiefly in areas over involved Purkinje cells, and desquamation. The pericardium and vessels were essentially negative.

Resurvey of nutritional status in Norris Point, Newfoundland. GRACE A. GOLDSMITH, WILLIAM J. DARBY, RUTH STEINKAMP (by invitation), ANNE STOCKELL (by invitation) and ELLEN MCDEVITT (by invitation). *Dept. of Medicine, Tulane Univ. School of Medicine, New Orleans, La.; Depts. of Biochemistry and Medicine, Vanderbilt Univ. School of Medicine, Nashville, Tenn.* Evaluation of the nutriture of 126 persons from 4 population segments—pre-school, pre-adolescent, mothers of children under 2 years, and other adults—was made in Norris Point, Newfoundland, in July, 1948. Following a comparable study in 1944 (J. Lab. Clin. Med. 30: 475) compulsory enrichment of flour was instituted. Dietary patterns were similar in the 2 studies, ¼ or more of the calories of half the subjects being derived from flour and cereals. Low intakes of ascorbic acid, calcium, vitamin A and riboflavin occurred in 25 to 50% of the examinees. The incidence of subjective complaints attributable to malnutrition was essentially the same in both surveys. Physical findings in 1948, as compared to 1944, showed somewhat less nasolabial seborrhea, thickening and pigmentation of pressure points, circumcorneal injection, neurologic changes, and a definite decrease in the incidence and severity of lingual lesions. The same or increased incidence was noted for cheilosis, conjunctival injection, dental caries, gingival changes, follicular keratosis and angular fissuring. Less conjunctival thickening was observed in pre-school children, an equal amount in other groups. Serum protein, hemoglobin, and vitamin A levels were generally satisfactory. Distribution of serum ascorbic acid was: 0.13, 0.39, and 0.65 mg/100 cc. for the first, second, and third quartiles, respectively. Four-hour urinary excretion, after an oral test dose of vitamins, was frequently low for riboflavin, but usually comparable to that in a group of normal laboratory subjects for thiamine and N′-methylnicotinamide. Correlations between dietary intake, physical signs, and laboratory findings will be presented and the significance of the data discussed.

Interrelation of age, serum cholesterol, and basal metabolism of women. MARY R. GRAM (by invitation) and RUTH M. LEVERTON. *Human Nutrition Research Laby., Univ. of Nebraska, Lincoln, Neb.* Serum cholesterol and basal metabolic rate were determined on 130 women between the ages of 18 and 70 years and living on self-chosen diets. Total serum cholesterol was determined on fasting blood samples by the Liebermann-Burchard reaction modified by Hoffman. Basal metabolic rate was determined with a Benedict-Roth respiration calorimeter and the results compared with normal values calculated on the basis of ideal weight by the method of DuBois as modified by Boothby and Sandiford. The women studied were in the following age groups: 18–25 yr., 52 subjects; 30–40 yr., 20 subjects; 40–50 yr., 28 subjects; 50–60 yr., 32 subjects; and 60–70 yr., 20 subjects. The mean serum cholesterol for each age group was 177.4, 200.8, 207.7, 250.6, and 257.4 mg. % respectively. Analysis of variance indicates this increase of cholesterol in serum with increasing age is significant. The mean basal metabolic rate for all women was 7% below the standard. The means of the basal metabolic rates for each age group were not significantly different. The relationship of basal metabolic rate to serum cholesterol and other blood values is being investigated for each age group.

Goiter in West Virginia. ISIDOR GREENWALD. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.* In 1928, Kimball, relying upon statements by 'older physicians' in Charlestown, claimed that goiter had been 'exceedingly rare' in the Kanawha valley before 1900 and that the high incidence in 1922 was due to replacement of local salt by a refined product, "the only change in food or water" that had occurred in the interval. The Journal of the American Medical Association has recently reiterated that claim (135, 434, 1947). The only information available regarding the iodine content of the local salt is given by Grimsley (West Virginia Geological Survey, Reports, vol. 4): the brine of the one well open in 1909 contained only one part of NaI per 61,400 parts of NaCl. Moreover, Drake, writing from 'among the upper waters of the Kanawha' in 1850, stated "Goitre. Cases are scattered here and there. I think they are not more numerous in proportion to the population than in the valleys of the Scioto and Cuyahoga" (Western Lancet 11: 558). Earlier reports of goiter in West Virginia are mentioned by Barton (Memoir Concerning Goiter, 1800). In 1893, goiter had disappeared from many parts of the country in which it had been endemic (Osler, Am. J. Med. Sci. 106: 503). Later, the disease reappeared. This sequence was well illustrated at Edmonton. There, too, an 'older physician' denied that the disease was present before 1900. However, cretins and large goiters were common in 1820 and many young girls had goiters as late as 1863. (Greenwald, Bull. His. Med. 17: 229, 1945.)

Effect of various amino acids on rats receiving niacin-tryptophan deficient rations. L. V. HANKES, L. M. HENDERSON (by invitation) and C. A. ELVEHJEM. *Dept. of Biochemistry, College of Agri-*

culture, Univ. of Wisconsin, Madison, Wis. Previous work has shown that 0.078% DL-threonine or 0.204% DL-phenylalanine will cause a growth inhibition in rats on a 9% casein sucrose ration deficient in niacin. The active isomers were found to be L-threonine and D-phenylalanine. When the 0.2% L-cystine, which is normally included in the ration was omitted, almost normal growth was obtained in the presence of 0.078% DL-threonine, 0.208% DL-phenylalanine, 2% acid hydrolyzed casein, or 2% glycine. However, the addition of 0.2% L-cystine with any of the above materials caused growth inhibition. When 0.2% DL-methionine replaced 0.2% L-cystine a growth inhibition occurred only with 0.078% DL-threonine or 2% acid hydrolyzed casein. Urine excretion studies indicated that acid hydrolyzed casein did not affect the amounts of N'methylnicotinamide or nicotinic acid excreted. DL-threonine (0.078%) in the presence of either L-cystine or DL-methionine did not affect the nicotinic acid levels in the urine. Since rats on a 9% casein dextrin ration showed a growth inhibition only with 6% gelatin, and not with 0.078% DL-threonine or 2% acid hydrolyzed casein, the effect of coprophagy on growth inhibition was studied. Six % gelatin caused a growth inhibition when the rats were in a square cage, but not when in a tube cage. Feces were analyzed for niacin.

Use of enriched cereals in certain diets consumed in the south. HARRIET A. HARLIN and BEULAH D. WESTERMAN (introduced by MARTHA M. KRAMER). *Dept. of Foods and Nutrition, Kansas Agr. Exp. Sta., Kansas State College, Manhattan, Kan.* Growth studies were made on young albino rats fed diets based upon the food consumed by certain low income groups in the South, to determine the significance of the use of enriched flour as a source of the B vitamins. The Negro tenants of the Mississippi Delta consume diets made up primarily of molasses, fat, cereals and black-eyed-peas with small quantities of eggs, vegetables, fruits and milk. When 58% of the calories in such a diet was derived from cereals, the growth of the rats consuming the diets containing enriched flour as the cereal component was greater than those consuming an equal quantity of non-enriched flour. When 45% of the total calories were derived from cereal, there was no difference in the growth gains made by the rats on the diets containing enriched and non-enriched flour. Certain people in South Carolina consume diets consisting of limited quantities of fat, meat, milk, eggs, vegetables, fruits and large quantities of cereals. When such diets were fed to rats and 50% of the total calories of the diet was furnished by enriched flour the animals with enriched flour in the diet made on the average 10 gm. greater growth gains than did those with non-enriched flour in the diet. When the enriched and non-enriched flour were fed at a level of 40 and 33% of the total calories in the diet no difference was noted in the average weight gains of the rats.

Concurrent feeding of amino acids. RICHARD HENDERSON (by invitation) and ROBERT S. HARRIS. *Nutritional Biochemistry Labs., Massachusetts Inst. of Technology, Cambridge, Mass.* Groups of weanling rats were fed a basal diet containing casein (30), corn starch (55.4), hydrogenated fat (10), tryptophan (0.17), histidine (0.13), saltmixture (4) and vitamin mixture (0.36). Control groups received the basal diet + 1% lysine. Test groups received the basal diet and the basal diet + 2% lysine at alternate feedings. The lysine replaced equal weights of corn starch in the diet. Groups were fed (1) one hour and fasted 7 hours, (2) one hour and fasted 5 hours and (3) one hour and fasted 3 hours throughout successive experiments lasting 27 days. A time-interval feeder was devised for the automatic opening and closing of the feeding jars. Significant differences were noted in nitrogen retention and in body weight gain, weight gain/gram of diet eaten, weight gain/gram of lysine eaten. These differences were greater as the feeding intervals lengthened. Thus a delay of 3 hours or longer, in the feeding of lysine as a supplement to a lysine-low diet interfered with metabolism. Lysine must be fed concurrently with other amino acids in the diet for most efficient utilization by the rat.

Vitamin B_{12} in swine nutrition. A. G. HOGAN and G. C. ANDERSON (by invitation). *Depts. of Animal Husbandry and Agricultural Chemistry, Univ. of Missouri, Columbia, Mo.* Six pigs were taken from their mothers at 2 days of age and transferred to cages with wide-mesh wire floors. They were reared on synthetic milk which included 'vitamin-free' casein, sucrose, corn starch, lard, mineral salts, and generous supplies of vitamins A, D, E, K, thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, choline, biotin, folic acid and inositol. Three of the pigs were given intramuscular injections of crystalline vitamin B_{12} (Merck) at 3-day intervals, until they were 38 days old. One pig received a total of 50, a second received 100, and the third received 200 mcg. All 6 pigs grew at about the same rate for the first 6 weeks. In the following 4-week period the pigs that received the injections made an average gain of 26.8 lb., the others gained 15.9 lb. In the following 6-week period one pig that did not receive vitamin B_{12} died, unexpectedly, and one has not gained consistently. The third began to decline and when injected with a total of 15 mcg. of vitamin B_{12} it began to gain at a moderate rate. The 3 that had received the vitamin injected grew at a uniform rate with an average gain of 58.8 lb., which is exceptional for that age. Three sows in all have been reared on synthetic diets. Two farrowed but were unable to rear their litters. The third never became

pregnant, presumably because of a constriction in each Fallopian tube.

Role of methionine in the protein metabolism of the rat. CECILE HOOVER (by invitation), PEARL SWANSON, and HELEN MERRIAM STEWART (by invitation). *Nutrition Laby., Foods and Nutrition Section, Iowa Agr. Exp. Sta., Iowa State College, Ames, Iowa.* Methionine, when added as a supplement to a nitrogen-poor diet offered to rats for a period of 21 days, exerts a marked body-sparing action. It has been postulated that in its absence, the body raids its own tissues to secure this compound for the fabrication of an essential metabolite(s). This hypothesis is being tested by studies of the partition of urine excreted by rats under various experimental conditions. The reduced excretion of urinary nitrogen resulting from the dietary addition of methionine is due to a marked decrease in both the total and relative amounts of urea nitrogen. Effects induced by the supplementary feeding of certain analogs of methionine also have been investigated. An accelerated catabolism, for example, accompanies the administration of ethionine. Although the quantity of nitrogen excreted is increased by 148 mg., the excretion of urea remains constant. Ethionine apparently acts like an antagonistic substance, thereby indicating that methionine occupies a key position is some metabolic reaction, and is important in this connection as a whole molecule rather than by virtue of specific constituent groupings. With caloric restriction, the elimination of fat from the test ration of rats results in a striking increment in urinary nitrogen due to increased quantities of urea. The addition of methionine averts the catabolic action of the low fat diet under these conditions. The ameliorative influence of dietary fat appears to rest on its ability to reduce the catabolism of compounds, the end products of which show up in the non-urea nitrogen fraction of urine.

Fatal lung hemorrhage and liver damage in rats prevented by vitamin E. E. L. HOVE, D. H. COPELAND (by invitation) and W. D. SALMON. *Laby. of Animal Nutrition, Alabama Polytechnic Inst., Auburn, Ala.* Young rats died suddenly after an average of 53 days on a Vitamin E-free, low-casein, high-fat diet. A sex difference in incidence was apparent: death came to 80% of the males (30/37) but only 30% of the females (3/10). No spontaneous deaths occurred among 28 litter mate controls receiving 7 mg. DL alpha tocopherol weekly. The diet contained water-washed casein 10, lard 19, cod liver oil 1, salt mixture 4, sucrose 66, choline 0.2, and an abundance of B vitamins. Necropsy revealed 3 major types of lesions: massive hemorrhage in the lungs; distension of small and large blood vessels of the body; and grossly evident liver damage which histologic section showed to be zonal necrosis. Evidence of Vitamin-E deficiency was shown by blanching of the teeth, testicular atrophy, creatinurea, and depressed growth (0.86 gm/day as compared with 1.26 gm/day for controls). Methionine, cystine or theophylline (0.1%) did not protect, although a higher casein level did. With 18% casein in the E-low diet, growth was optimum, and the rats remained in apparent good health for a year. The protective factor in casein could be destroyed by hydrogen peroxide. Death and liver damage occurred in 5 of 6 rats on E-low diets containing 18% oxidized casein fortified with tryptophane and methionine. The DL-methionine requirement for maximum growth was over 1% although this level did not protect against the acute Vitamin-E deficiency.

Influence of vitamin E on the creatine and creatinine excretion of rats poisoned with CCl_4. E. L. HOVE. *Laby. of Animal Nutrition, Alabama Polytechnic Inst., Auburn, Ala.* A sharp increase in creatine excretion and a reduction in creatinine excretion has been observed in rats poisoned with CCl_4. The same condition occurs as the result of a chronic vitamin-E deficiency. Therefore a study was made of the influence of vitamin E on the creatine-creatinine excretion pattern following CCl_4. Groups of rats on a 10% casein, E-low diet were fed 0, 0.5, 2.5 and 12.5 mg. of DL, alpha tocopherol daily for 10 days prior to injections of single doses of 1 cc. of CCl_4/kg. body weight. The maximum creatine excretion level, expressed as mg/rat/day, was 25 for the E-free group, but only 7 for the group fed the highest tocopherol level. The fall in the creatinine excretion was delayed in the tocopherol fed group and the return to normal values was more rapid. The lower tocopherol levels gave intermediate values. Half of the E-free control group died as the result of CCl_4. None of the tocopherol-fed rats died. The values given for the creatine-creatinine excretion of the E-free group are based on survivors.

Role of the gastrointestinal tract in the conversion of tryptophan to nicotinic acid. JAMES M. HUNDLEY (introduced by F. S. DAFT). *Experimental Biology and Medicine Inst., National Inst. of Health, Bethesda, Md.* Several investigators have advanced evidence indicating that the intestinal bacteria can supply a part or perhaps all of the requirement for nicotinic acid in certain species. Since tryptophan is known to be a dietary precursor of nicotinic acid, the idea has been advanced that intestinal bacteria may convert tryptophan to nicotinic acid. To test this possibility, we have studied the efficiency of conversion of tryptophan to nicotinic acid in rats before and after surgical removal of the gastrointestinal tract. The amount of urinary N′-methylnicotinamide (NMN), expressed as γ per 100 gm. of rat in 24 hours, was used as an index of the amount of nicotinic acid synthesized. 100 mg. of L(-)tryptophan given subcutane-

ously in 10 cc. of saline was the test dose in all instances. 26 male rats weighing about 250 gm. each were maintained on a basal purified 'nicotinic acid free' diet. The rats excreted 120 γ of NMN on this diet alone. Tryptophan increased the excretion to 1274 γ. After surgical removal of the entire intestine, excepting the stomach, the basal excretion of NMN was 246 γ. Tryptophan increased the excretion to 2156 γ. After gastrectomy only, the basal excretion was 182 γ which tryptophan increased to 1864 γ. These data have been interpreted to mean that neither the stomach nor the bacteria of the intestinal tract are involved in the synthesis of nicotinic acid from tryptophan.

Comparison of sugars in the purified diet of baby pigs. S. R. JOHNSON. *Dept. of Animal Industry, Univ. of Arkansas, Fayetteville, Ark.* Five litters of baby pigs were fed 'synthetic' milk in which all nutrients were in purified form. When the carbohydrate was sucrose, acute diarrhoea quickly developed, resulting in rapid weight loss and death within 48 hours unless a ration change supervened. Hemorrhagic kidney syndrome, probably like that observed in young rats on low choline sucrose-base diets, resulted from the use of sucrose. When the ration was quickly changed to cows' milk, recovery occurred. If the pigs were started on cows' milk and changed very gradually to the sucrose-base diet, they tolerated the sucrose. Addition of 16% yeast, 0.4% d-l methionine, or a double allowance of pure vitamins did not prevent the sucrose syndrome. Glucose ('Cerelose') instead of sucrose in the purified diet resulted in uneventful growth with little or no diarrhoea or kidney damage. Lactose was satisfactory in the starting ration but pigs on the lactose-base diet eventually developed chronic diarrhoea, grew more slowly than those receiving glucose, and one pig continued on the lactose diet developed probable biotin deficiency. Levulose was unsatisfactory as the carbohydrate (2 cases), and addition of sucrase enzyme to the sucrose-base diet did not improve performance (2 cases). Baby pigs could be started very successfully with only one small nursing of colostrum, cows' milk while learning to drink the first day, then with the purified diet with either glucose or lactose as the carbohydrate.

Perspiration as a factor influencing the iron requirement. FRANCES A. JOHNSTON and GLADYS C. HAGAN (by invitation). *N. Y. State College of Home Economics, Cornell Univ., Ithaca, N. Y.* In studies of the iron requirement the loss of iron in perspiration is not usually taken into consideration. Unless the loss is extremely small it will raise the requirement appreciably because of the low percentage of iron absorbed from the diet. The problem of collecting perspiration free of contamination is so difficult that all we could do was obtain a value which would set a top limit to the amount of iron that might be present. The iron content of filtered water collected from washing 2 subjects after perspiring for 2 hr. at 100°F. and 59–67% relative humidity was 0.02, 0.02 mg. respectively. This would amount to 0.24 mg. for 24 hr. of profuse perspiration. Since perspiration seldom continues at a profuse rate for 24 hr., a loss as large as this would be unusual. Perspiration induced by high temperatures and humidity and collected without dilution was found to contain a mean value of 0.06, 0.05, 0.03, 0.02 and 0.04 mg/l. for each of 5 subjects. Except under extreme conditions of temperature, humidity or activity, the excretion of perspiration probably does not exceed 1 l/day. Thus, under normal living conditions in the north temperate zone, the loss of iron in perspiration is negligible and probably does not exceed 0.02 to 0.06 mg/day. The possibility that perspiration contains no iron can not be ignored. In 3 of the 19 samples collected from the 5 subjects no iron was present.

Color measurement in products of rice milling. MARINUS C. KIK. *Univ. of Arkansas, Fayetteville, Ark.* Whole kernel samples of mill products (brown, first break, second break, and head or white rice) of Blue Rose, Zenith, California Pearl and Nira, tested for color with the Hardy self-recording photoelectric spectrophotometer, gave average percentage reflectances at 460 mμ and at 660 mμ: for brown rice, 25 and 51; for first break, 33.6 and 59.2; for second break, 36.2 and 61.3 and for head rice, 36.1 and 60.6. In the whole visible region (especially in the yellow), reflectance values between brown and white rice differed considerably, were small between first break and nonappreciable between second break and white. The photovolt photoelectric reflectometer, with blue, green and red filters, gave similar results. Blue and green filters were more effective than the red filter. In these latter studies rice samples with different bran removal (1 to 10%) were tested for color. The widest spread of readings between samples of one variety is obtained when measurements are made with 'suppressed zero.'.Using the blue filter and for brown rice a setting at 0, gave readings for first break, second break and head rice of 50, 58 and 69 respectively. The results of these reflectometer studies might find application in government grading, in description of standards for rice and in controlling rice milling to a specific degree of whiteness.

Pteroylglutamic acid studies on patients with tropical sprue: Factors affecting urinary excretion levels. ELIZABETH M. KNOTT and RAMÓN M. SUÁREZ (introduced by R. R. WILLIAMS). *School of Tropical Medicine, San Juan, Puerto Rico.* The urinary excretion of pteroylglutamic acid has been assayed with Lactobacillus casei, for 313 24-hour specimens and 237 2–8-hour collections, from 65 individuals. Patients with tropical sprue were diagnosed in accordance with the criteria defined by

Suárez, et al. (*Ann. Int. Med.* 26: 643, 1947). Results are reported as micrograms. The average total daily excretion for sprue patients was 1.109 (range for 6 patients 0.166–0.778, and 4 patients 1.180–3.068). Eight staff members averaged 1.693 (range 0.920–4.100). Two non-sprue, anemic patients excreted 0.418 and 0.243 on a low protein diet, increased to 0.650 and 0.433 when niacin was added, and increased to 1.544 and 1.273 when given the regular hospital diet. Three anemic patients on a diet high in protein and vitamins, plus injections of liver extract, excreted an average of 4.340 (range 3.909–4.715). The night rate of excretion, in μg/hr., was 0.022 for sprue patients, 0.057 for other hospital patients, and 0.076 for staff members. During fasting in the morning, the rate of excretion decreased for sprue patients to 0.016, but increased to 0.094 and 0.119 for the other hospital patients and staff members. One staff member, although receiving an excellent diet, excreted amounts of pteroylglutamic acid comparable to the lower figures for sprue patients, and had a tendency for repeated episodes of a spruelike syndrome. Women during the last trimester of pregnancy had night excretion rates of 0.068 and fasting rates of 0.101, but decreased to 0.049 and 0.091 during the first week postpartum.

Paradoxical relationship between serum level and liver content of vitamin A. R. F. KRAUSE (introduced by H. B. PIERCE). *Dept. of Biochemistry, College of Medicine, Univ. of Vermont, Burlington, Vt.* An examination has been made of the relationship between serum level and liver content of vitamin A in normal rats and those under the influence of varying degrees of vitamin-A depletion. An inverse relationship has been found between the blood and liver levels of vitamin A in normal rats and in depleted animals whose liver content ranged as low as 600 IU/total liver. However, under conditions of more severe depletion when the total liver content fell below 600 IU, there was a parallelism between blood and liver levels. This evidence supports the hypothesis that there is a regulatory mechanism controlling blood vitamin-A levels of the rat which, under the conditions described above, operates to give an inverse relationship between blood level and liver content of vitamin A. This mechanism, however, fails to function under conditions of extreme depletion.

Some inter-relationships of thiamine and fat in the nutrition of the rat. M. M. KRIDER (by invitation) and N. B. GUERRANT. *Dept. of Agricultural and Biological Chemistry, Pennsylvania State College, State College, Penna.* Studies, with regulated intakes of isocaloric diets, designed to clarify the probable inter-relationship of the thiamine and fat intake of the rat and the amounts of thiamine and fat stored in the rat's body have yielded data that permit the following conclusions: *1*) Increasing the thiamine intake from 2 to 1000 μg. daily had less effect on increasing the body-weights of rats than did increasing the fat content of the diet from 0 to 20%. This increase in body-weight was due primarily to greater fat deposition attained by the rats receiving the fat-containing diets. *2*) The cardiac thiamine of rats was found to depend primarily on the thiamine intake and was not markedly affected by the fat content of the diet. *3*) Under the conditions of the experiments, the fat content of the rat's body was found to increase with the fat content of the diet and was not markedly influenced by the thiamine intake where the intake of this vitamin was 50 μg. or more/day. *4*) The index of refraction of the body fat of rats was found to vary with the fat (Crisco) content of the rat's diet and to decrease with an increase in the amount of fat present in the rat's body.

Effect of dietary restriction and rehabilitation upon the spontaneous activity of rats. HARRY KRZYWICKI and ESTHER DA COSTA (introduced by ROBERT E. JOHNSON). *U. S. Army Medical Nutrition Laboratory, Chicago 9, Ill.* During an average of 14 weeks, a control group of 12 male rats fed ad libitum gained 121% in weight with an average food intake of 14.9 gm/rat/day and showed an average activity of 1101 rotary cage revolutions/rat/day. Eighteen rats fed a quantitatively restricted but otherwise complete diet lost 42% in weight with a food intake of 8 gm/rat/day and an average activity of 7854 cage revolutions/rat/day. The peaks in activity of the restricted rats occurred during the 6th, 9th and 10th weeks. Six rats fed 8 gm/rat/day of a low protein, high salt diet lost 32% in weight and showed an activity of 3351 cage revolutions/rat/day. During dietary rehabilitation, spontaneous activity decreased promptly and showed an average of 1143 revolutions/rat/day with a high fat diet, 1176 revolutions/rat/day with a high carbohydrate diet and only 640 revolutions/rat/day for a high protein diet. Thus both types of food restriction caused increased activity but different types of refeeding led to significantly different reductions of spontaneous activity.

Nitrogen excretion of women related to the distribution of animal protein in the daily meals. RUTH M. LEVERTON and MARY R. GRAM (by invitation). *Human Nutrition Research Laboratory, Univ. of Nebraska, Lincoln, Neb.* This study is a preliminary investigation of whether the time element in the feeding of complete proteins affects the utilization of the nitrogen by human subjects. Fourteen college-age girls were kept on a constant diet which furnished 62 gm. of protein daily for 36 days. During the first 18-day period a fruit-bread products-coffee breakfast, which furnished no protein from animal sources, was served and all of the animal protein was given in the noon and evening meals. During the second 18-day period a glass of milk was taken from the evening meal and added

to the breakfast. Metabolism data were secured on all subjects for the last 9 days of each of the 2 periods. The average daily excretion of nitrogen for all subjects for the first and second periods was 9.07 and 8.57 gm. respectively for urinary nitrogen, and 0.57 and 0.54 gm. respectively for fecal nitrogen. Differences between daily nitrogen excretion of the 2 periods were not always statistically significant. On the same level of protein intake there was, however, a persistent trend toward a lower nitrogen excretion when the animal protein from a glass of milk was in the breakfast than when the animal protein was present only in the noon and evening meals. All subjects were in nitrogen equilibrium during the first period and retained an average of 1.03 gm. nitrogen daily during the second period.

Studies involving protein stores in man. STANLEY LEVEY, JOHN E. HARROUN and CHARLEY J. SMYTH (introduced by ARTHUR H. SMITH). *Wayne County General Hospital, Eloise, Michigan, and the Depts. of Physiological Chemistry and Medicine, Wayne Univ. College of Medicine, Detroit, Mich.* The plasma volume (dye method), total circulating proteins and albumin were determined before and 30 minutes after the infusion of 1000 ml. of physiological saline in 4 normal, healthy males and in 6 male patients exhibiting various degrees of undernutrition. The undernourished subjects were then placed on a high caloric (4000–5000), high protein (2.0 to 4.0 gm/kg/day) diet for a period of at least 3 weeks after which the above procedure was repeated. The subjects with normal nutrition demonstrated a constant response to the saline infusion characterized by an increase in plasma volume, approximately 350 ml., a decrease in serum protein concentration, but an increase in total circulating proteins. The albumin changes did not follow a consistent pattern. The response to the saline infusion exhibited by the malnourished patients was characterized by decrease in both the protein concentration and in the total circulating proteins, in spite of an increase in plasma volume. The malnourished subjects who were followed through the controlled dietary period demonstrated an increase in circulating proteins after the saline infusion similar to the normal controls. The major interest of this study centers on the changes in the circulating proteins following the saline infusion. The increase in circulating proteins demonstrated by the normal patients is explained by a washing into the circulation of proteins from a labile protein store. The decrease in circulating proteins exhibited by the undernourished patients is explained by a lack of labile protein stores. The change from the malnourished to the normal pattern after the period of dietary treatment demonstrates the repletion of the protein stores.

Human urinary and fecal elimination of vitamin B_6 and 4-pyridoxic acid on a controlled diet. HELLEN LINKSWILER and MAY S. REYNOLDS (introduced by HELEN T. PARSONS). *Dept. of Home Economics, Univ. of Wisconsin, Madison, Wis.* The pyridoxine intake and the pyridoxine and 4-pyridoxic acid elimination by 12 normal human subjects on a controlled dietary intake have been determined. The diet furnished from 0.5 to 1.0 mg. of B_6 and from 19 to 32 gm. vegetable protein per day by analysis. Supplements of thiamine, riboflavin, niacin and calcium lactate were given. Calories were adequate to maintain weight. A period of 9 days with no pyridoxine supplement was followed by a 9-day period during which 15 mg. pyridoxine per day were given. In the first period, the pyridoxine and 4-pyridoxic acid eliminated ranged from 0.85 to 3.7 mg. in excess of the pyridoxine intake. 4-pyridoxic acid accounted for approximately 80% of this total excretion. Intestinal synthesis of B_6 in man has been assumed by other investigators but has not been previously demonstrated. Studies of the effect of sulfasuxadine on synthesis are in progress. When the diet was supplemented with 15 mg. of pyridoxine HCl daily only 50 to 60% of the total intake was eliminated. 4-pyridoxic acid excretion during this period ranged from 75 to 80% of the total metabolites determined.

Age and calcium utilization. C. M. MCCAY, F. E. LOVELACE (by invitation), C. H. LIU (by invitation) and G. KANE (by invitation). *Laboratory of Animal Nutrition, Cornell Univ., Ithaca, N. Y.* Rats, starting at 38 days of age stored 90% of the calcium from a diet containing 0.3% Ca, until the animals were 110 days old. At 160 days of age these rats stored 55–65% of the Ca. Littermate rats fed a similar diet containing enough oxalate to tie up $\frac{3}{4}$ of the dietary Ca stored only the free Ca until 70 days of age. After this age the utilization improved until 80% of all dietary Ca was stored at 100 days of age. After this Ca storage was only 10% below the controls in spite of the presence of oxalates. If oxalates were fed with meat in the morning and calcium fed as milk 5 hours later, the utilization of Ca was improved during the months shortly after weaning, when assimilation of Ca was difficult. Analogous studies with young puppies gave similar results. In order to determine the effect of the lipids of the diet upon the wastage of dietary calcium in old rats, balances were run upon animals ranging from 450 to 900 days of age. On a weight basis the fat in the diet was varied from 2 to 30%. Under conditions of a constant but marginal daily intake of Ca, the higher fat levels created a greater negative balance. A similar effect was found when the percentage of Ca in the diet was kept constant but the Ca ingested was increased, due to the consumption of more of the low fat food.

Effect of carbohydrate on the nutritive value of heated protein. IVAN J. MADER (by invitation), LAWRENCE J. SCHROEDER (by invitation) and

ARTHUR H. SMITH. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.* A nitrogen balance study was used to determine the possible role of carbohydrate in altering the nutritive value of heated protein. A synthetic diet containing all the known dietary essentials was fed to three adult female dogs. Commercial and 'carbohydrate-free' lactalbumin, heated under various conditions, served as the protein nitrogen sources. All diets fed were isocaloric and daily nitrogen intakes kept at a constant minimal level for each dog. Based on the partition of nitrogen excretion between the urine and feces, no changes in digestibility or nutritive index occurred when the protein was dry-heated at 120°. Using the original lactalbumin as a criterion, significant decreases in both digestibility and nutritive value did occur when the lactalbumin was autoclaved. This decrease in nutritive value was proportional to the severity of heat treatment. Commercial lactalbumin contains large amounts of reducing substances, chiefly lactose. Water extraction allowed almost complete removal of the carbohydrate. The extracted lactalbumin, heated in the same manner as the commercial lactalbumin, was fed in another series of experiments. After removal of the carbohydrate, an increase was observed in both the digestibility and nutritive index of the autoclaved protein. The extracted lactalbumin was reconstituted with lactose with respect to the carbohydrate content of the original protein. Upon heating, the carbohydrate content diminished and severe negative nitrogen balances resulted. Decreased digestibility of the autoclaved lactalbumin, as evidenced by increased fecal nitrogen, accounted for the decrease in its nutritive value. Utilization of absorbed nitrogen remained uniformly high in all cases.

Effect of various degrees of starvation on gonadotrophic and lactogenic content of rat pituitaries. JOSEPH MEITES and J. O. REED (introduced by C. F. HUFFMAN). *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing, Mich.* Five groups each of intact and ovariectomized mature rats were put on full, ¾, ½, ¼ and no-feed regimes. The first 4 groups were killed at the end of 14 days and the 5th group at the end of 7 days. Pituitary gonadotrophic content was determined by injecting an aqueous aliquot of 5 pituitaries from each group into 5 immature female rats during a 5-day period, and weighing the ovaries and uteri on the 6th day. Lactogenic potency was measured by injecting an aqueous aliquot of 2 pituitaries from each group intradermally over the crop glands of 5 pigeons during a 4-day period, and rating the crop responses on the 5th day. Regardless of the degree of starvation in either intact or ovariectomized rats, there was no reduction in pituitary gonadotrophic content. This occurred despite the marked atrophy of the ovaries and uteri of the intact rats, and the high initial pituitary gonadotrophic content of the ovariectomized rats. Pituitary lactogenic potency was reduced in both intact and ovariectomized rats on the ½, ¼ and no-feed regimes, corroborating the report of Meites and Turner (*Mo. Agr. Exper. Sta. Res. Bul.* 416, 1948) that mother rats starved during lactation show a decrease in pituitary lactogenic content. It is concluded that during starvation, pituitary gonadotrophic content is not and pituitary lactogenic content is an accurate index of the amount of hormone released into the blood stream.

Folic acid deficiency and the respiration and pyruvate utilization of duckling erythrocytes and cardiac muscle. O. NEAL MILLER (by invitation), ROBERT E. OLSON (by invitation) and FREDRICK J. STARE. *Dept. of Nutrition, Harvard School of Public Health, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Folic acid deficiency was induced in 4-day-old white Pekin ducklings by feeding a purified diet supplemented with sulfaguanidine. After the onset of anemia, the deficient birds were taken for respiration studies by the direct method of Warburg. Controls fed the deficient diet plus folic acid were tested simultaneously. Blood was drawn from the jugular vein into heparinized syringes, centrifuged, and washed once with cold phosphate-saline at *p*H 7.4. Slices of heart ventricle from the same birds were prepared. The washed red cells and the cardiac muscle slices were then incubated at 37°C. for 1 hr. after equilibration in an atmosphere of oxygen. When pyruvate was added as substrate, it was initially present in the medium. The disappearance of pyruvate and formation of lactate was determined chemically and the net pyruvate utilization, i.e., the conversion of pyruvate to non-lactate products, calculated. Data obtained with heart muscle slices are expressed in terms of (Q) while that obtained with the red cell suspensions are expressed in terms of microliters of substrate or oxygen utilized per ml. of standard blood (hematocrit = 38). Slices of ventricle from folic acid deficient ducks showed no depression in oxygen consumption below that of controls either with added pyruvate (Q_{O_2} = 12) or without pyruvate (Q_{O_2} = 9). The conversion of pyruvate to lactate, however, was increased, and the conversion of pyruvate to non-lactate products was decreased approximately 20% by the deficiency. The erythrocytes from deficient birds showed a decrease in both respiration and pyruvate utilization.

Fate of ascorbic acid, dehydroascorbic acid and diketogulonic acid in the animal body. MARY B. MILLS (by invitation), CHARLOTTE M. DAMRON (by invitation) and JOSEPH H. ROE. *Dept. of Biochemistry, School of Medicine, George Washington Univ., Washington, D. C.* Normal guinea

pigs maintained on a stock diet supplemented with cabbage were injected or fed by stomach tube with pure solutions of ascorbic acid, freshly prepared dehydroascorbic acid, or a calcium diketogulonate. After 1 or 2 hours, the animals were sacrificed and the various tissues analyzed for ascorbic acid, dehydroascorbic acid, and diketogulonic acid. Ascorbic acid was recovered as such to the extent of about 40–50% of the amount administered. Dehydroascorbic acid was recovered to about 30–40% of the amount given and some was reduced to ascorbic acid or converted to diketogulonic acid in the animal body. Diketogulonic acid was recovered to about 25–30% of the amount given and there was no evidence of transformation to the other two compounds being analyzed. Young guinea pigs about 50–100 gm. in size were used as a check upon the total recoveries obtained with adult pigs. The animals were injected with the test compound and killed 1 or 2 hours after injection. The animal was skinned and the carcass was ground into a slurry in the Waring blendor under 10% stannous chloride solution containing CO_2. The slurry was made to a satisfactory volume with 5% HPO_3 and analyzed for total ascorbic, dehydroascorbic and diketogulonic acids. Recoveries were a little higher with ascorbic and dehydroascorbic acids, and a little lower with diketogulonic acid, than those obtained with adult animals. The data obtained with young guinea pigs confirmed the observations on the fate of the three compounds in adult animals.

Essentiality of vitamin B_{12} for the baby pig, with preliminary quantitative data. A. L. Neumann (by invitation), Marian F. James (by invitation), J. L. Krider (by invitation) and B. Connor Johnson. *Dept. of Animal Science and Division of Animal Nutrition, Univ. of Illinois, Urbana, Ill.* Isolated soybean protein 'synthetic milk' fed to baby pigs was improved by supplementation with a vitamin B_{12} concentrate, both from the standpoint of growth and of physiological well-being. Forty-two micrograms of vitamin B_{12}/kg. dry matter in the diet produced the maximum growth response and improved hematopoiesis. Grossly, the vitamin B_{12} deficient pigs were extremely irritable, sensitive to touch, sluggish in their movements, and a number of the pigs were unable to stand upon their rear legs. All the vitamin B_{12} pigs on the two highest levels fed grew better and showed none of the above symptoms. One group fed a manure factor concentrate showed the same gross symptoms as the vitamin B_{12} deficient pigs but grew almost as rapidly as those fed the higher levels of vitamin B_{12}. Hematopoiesis was rather severely strained in all the pigs fed the B_{12} deficient diets as well as in those fed the manure factor, as indicated by bone marrow and blood studies, even though 0.4 gm. pteroylglutamic acid was included per kg. dry matter in the diet. Histological information on the nerves, gastro-intestinal tract and other organs will be presented, as well as data on the blood and bone marrow condition. The growth effect of the injection of crystalline vitamin B_{12} in baby pigs fed the same basal diet will also be reported as well as some preliminary data on a pteroylglutamic acid vitamin B_{12} relationship.

Biochemical studies on primigravidae on self-selected diets differing greatly in nutritive value. Helen Oldham, Bernice Blum Sheft (by invitation) and Thelma Porter. *Dept. of Home Economics, Univ. of Chicago, Chicago, Ill.* Primigravidae, living at home on self-selected diets, were studied for 10-day periods during each of the last 5 months of pregnancy. All food eaten during the first 7 days of each period was weighed and samples were saved for thiamine, riboflavin, niacin, nitrogen and tryptophane analyses. Intakes of other nutrients were calculated. Complete collections of excreta were made during the 7 days and aliquots analyzed. On the 7th, 8th, 9th and 10th days, after identical breakfasts, 4-hour urine specimens were collected. That on the 7th day served as a control and those on the 8th, 9th and 10th days as a measure of riboflavin, niacin and thiamine test doses, respectively, which were given with breakfast. The average daily intakes of the different subjects ranged as follows: calories, 400 to 2300; protein, 10 to 100 gm.; Ca, 0.1 to 2.7 gm.; iron, 4 to 150 mg.; vitamin A, 600 to 16,600 IU; vitamin D, 500 to 3000 IU; ascorbic acid, 6 to 160 mg.; thiamine, 0.35 to 19 mg.; riboflavin, 0.23 to 20 mg.; niacin, 4 to 86 mg. High intakes of vitamins and minerals were due for the most part to voluntary supplementation; the low, to poor dietary habits. The proportion of the thiamine intake which was excreted was relatively constant whereas that of riboflavin varied directly with the intake. Test dose returns of both thiamine and riboflavin were very low on low intakes. Excretion of niacin metabolites on low intakes usually exceeded the amount of niacin ingested, sometimes by as much as 100%. Niacin test dose returns of these subjects also tended to be high. Consistently negative nitrogen balances were found only when the average daily protein intakes were less than 30 gm.

Metabolism of radioactive acetate and pyruvate by cardiac muscle from normal and pantothenic acid-deficient ducklings. Robert E. Olson (by invitation) and Fredrick J. Stare. *Dept. of Nutrition, Harvard School of Public Health, and Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass.* Acetate labeled in the carboxyl with C^{14} and 2 isomers of pyruvate, labeled respectively in the carboxyl group and the alpha carbon atom were synthesized. Slices and dilute homogenates of heart ventricle from normal and

pantothenic acid-deficient ducklings were incubated with these substrates in Warburg flasks. The gas phase in the slice experiments was O_2; in the homogenates, air. Total pyruvate disappearance and lactate appearance were determined chemically and the conversion of pyruvate to non-lactate products expressed as net $-Q_{pyruvate}$. The rate of oxidation of the labeled carbon atom in the respective substrates was determined by counting the $C^{14}O_2$ trapped in the center well. All substrates were tipped in from side arms after equilibration and gave a final concentration of 5 mM/l. The following representative data were obtained in a series of normal ducklings:

	Net $-Q$ Pyruvate	$Q_{C^{14}O_2}$ Pyruvate Carboxyl	$Q_{C^{14}O_2}$ Pyruvate α-carbon	$Q_{C^{14}O_2}$ Acetate Carboxyl
Slice	5.2	2.8	1.8	1.1
Homogenate	3.8	3.7	2.2	0.7

These data indicate that in the slice, but not in the homogenate, considerable amounts of added pyruvate are metabolized to non-lactate products without the loss of CO_2. The oxidation of added pyruvate in slices of ventricle from pantothenic acid-deficient ducks was essentially normal. In homogenates of deficient ventricle, however, there was a marked depression in pyruvate oxidation. The oxidation of the alpha-carbon atom was reduced more than the rate of decarboxylation. The oxidation of acetate was reduced in both slices and homogenates.

Relationship of riboflavin deficiency to adrenicortical function as shown by carbohydrate metabolism in anoxia. BLUEBELL READE (by invitation) and AGNES FAY MORGAN. *Laby. of Home Economics, Univ. of California, Berkeley, Calif.* Young rats were subjected fasting to anoxic anoxia after 1, 3, 5, 8 and 12 weeks on riboflavin-deficient diet. After the test period of 24 hours, blood sugar, liver glycogen and muscle glycogen were determined. Comparable animals kept at sea-level under the same conditions were similarly examined, as were rats given the same diet plus riboflavin under anoxia and at sea-level. The total carbohydrate content of the deficient animals was greater than that of those on normal diet through the third week but this decreased rapidly below the normal fasting level in the 5th to the 12th week. Injection of 0.1 mg. riboflavin immediately before, or of 2 ml. cortin in the 24 hours just preceding the test period, restored the carbohydrate-producing capacity of the deficient rats. Injection of desoxycorticosterone, 5 mg. in oil, in the 48 hours preceding the test had little effect. Adrenalectomized rats on the control diet were not enabled to increase their carbohydrate content by injections of 0.1 mg. riboflavin. Pair-fed normal controls exhibited increased gluconeogenetic capacity above that of the full-fed positive controls. The behavior of the deficient animals corresponded with that described by Selye as 'alarm reaction' during the first part of the deficiency and 'collapse' in the latter part. This may indicate that the state of riboflavin deficiency involves increased adrenicortical activity at first followed by adrenal cortex exhaustion. Either the complete cortical extract, cortin, or riboflavin substituted for, or restored adrenicortical function.

Relation of age to ascorbic acid metabolism in guinea pigs. MARY ELIZABETH REID. *Expt'l. Biol. and Med. Inst., N. I. H., Bethesda, Md.* Two groups of inbred male guinea pigs, one slightly over 2 months of age and the other ranging from 1 to over 3 years, were fed a commercial pelleted diet, supplemented with 1% of powdered yeast, vitamin E, and a small amount of fresh carrots. Daily intraperitoneal injections of ascorbic acid (5 mg/100 gm.) were given for at least 3 weeks before the experiments were made. At periods varying from ½ to 24 hours after the usual daily injection, blood samples were taken, following which the animal was killed. The liver, kidneys, spleen, adrenals, and the contents of the gastrointestinal tract were removed, assayed for ascorbic acid and the responses of the young and old groups to the experimental procedure were compared. The basal, pre-injection levels in the blood and liver were found to be lower in the young animals. Following administration of the vitamin, the levels in both groups rose rapidly in the blood, kidneys, and liver but showed no consistent variations in the adrenals and spleen. The rapidity of increase and the subsequent return to basal levels varied from organ to organ. The young animal showed a more rapid increase in the liver content but it also decreased earlier. The kidneys of the young animal maintained a high level much longer than the older group suggesting the possibility of greater tubular reabsorption. Assay of the contents of the digestive tract yielded no evidence of more rapid excretion and destruction in the younger group. It is known that the dietary requirements of vitamin C for young and old animals is approximately the same per unit of weight. However, these present results together with the results of earlier studies suggest that the younger animal actually stores and uses more vitamin C than the older animal. The evidence indicates that there is greater wastage in the older animals.

Germ-free chick nutrition: development on synthetic-type diets. J. A. REYNIERS (by invitation), T. D. LUCKEY (by invitation), P. C. TREXLER (by invitation), R. F. ERVIN (by invitation), M. WAGNER (by invitation), H. A. GORDON (by invitation). *Germ-Free Life Division, LOBUND,*

Notre Dame, Indiana; R. A. BROWN, G. J. MANNERING (by invitation) and C. J. CAMPBELL (by invitation). *Research Labys., Parke, Davis & Co., Detroit, Mich.* The role of intestinal microorganisms in nutrition research is of considerable theoretical and practical importance. Attention has been largely directed toward the possible synthesis of vitamins by the intestinal flora. This report records a study to determine whether germ-free chicks require a dietary source of unknown vitamins. Chicks were obtained and reared germ-free according to the method of Reyniers *et al.* (LOBUND Reports, No. 2). The animals were fed autoclaved water and synthetic-type diets (with and without liver and yeast) for 4 to 9 weeks. Bacteriological tests (microscopic and cultural) showed the chicks to be free from all demonstrable living microorganisms. The diets used were adequate as shown by chemical and biological methods. The percentage of nutrients lost during autoclaving at 122°C. for 25 minutes is as follows: thiamin, 90; riboflavin, 15; niacin, 3; pantothenic acid, 45; pyridoxine, 31; biotin, 0; choline, 12; folic acid, 64; vitamin A, 19; vitamin E, 46; vitamin K, 38; lysin, 0; tryptophane, 8. Feather development was excellent in the germ-free chicks. When 4 weeks old, they were somewhat heavier than the control chicks fed the same diet in 4 of 7 experiments. The presence of liver and yeast powder or fresh liver extract did not significantly increase growth rate or hemoglobin formation. Hematopoiesis was normal except for some deviations in the white blood cell picture. Perosis occurred in some of the germ-free birds. The results indicate that the nutritional requirements of germ-free chicks are similar to those of the controls. Therefore if the search for unknown vitamins is to be carried further, it is evident that either the diets or the animals used will have to be changed.

Amino acids and vitamin B_{12} as supplements to plant proteins for growing chicks. L. R. RICHARDSON, L. G. BLAYLOCK (by invitation), H. L. GERMAN (by invitation) and R. M. SHERWOOD (by invitation). *Texas Agricultural Experiment Station, College Station, Texas.* Amino acids and vitamin B_{12} were investigated as supplements to commercial cottonseed meal low in gossypol and to soybean meal for growing chicks. The protein concentrates were added in quantities to supply a total of 20% protein in the diet. One typical diet contained corn meal, protein concentrate, steamed bone meal, manganese sulfate and all vitamins required by growing chicks. Another contained cerelose instead of grain and mineral mixture instead of bone meal. The experimental period was 4 weeks. Chicks receiving the corn cottonseed meal diet weighed an average of 150 gm. while those receiving the same diet plus 0.5% of L(+) lysine weighed 256 gm. When the basal diet contained 3% sardine meal the chicks weighed an average of 206 gm. without and 252 gm. with lysine. The further addition of 0.1% of DL-tryptophan and 0.2% of DL-methionine to the cottonseed meal sardine meal diet increased the weight to 282 gm. A vitamin B_{12} concentrate was investigated as a supplement to cottonseed and soybean meals. Three chicks receiving a cerelose cottonseed meal diet supplemented with 0.8% of DL-lysine in addition to tryptophan and methionine weighed an average of 228 gm. while 3 receiving the same diet plus 40 Mcg/kg. of vitamin B_{12} weighed an average of 300 gm. Two groups of 17 chicks each receiving the cerelose soybean meal diet weighed an average of 250 gm. without and 285 gm. with vitamin B_{12}.

Metabolism of p-aminobenzoic acid and some of its conjugates by the rat. THOMAS R. RIGGS (by invitation) and D. MARK HEGSTED. *Dept. of Nutrition, Harvard School of Public Health, Dept. of Biological Chemistry, Harvard Medical School, and Dept. of Biochemistry and Nutrition, Tufts College Medical School, Boston, Mass.* The acetylation of p-aminobenzoic acid (PAB) in rats has been shown to be diminished in pantothenic acid deficiency (*J. Biol. Chem.* 172: 539, 1948) and also to be influenced by deficiencies of thiamine and riboflavin and by the amount of PAB administered (*J. Biol. Chem.* in press). Since PAB can be conjugated at the carboxyl group (with glycine) as well as at the amino group, it appeared that the extent of acetylation of the amino group would probably be influenced by the extent of conjugation of the carboxyl group. Determinations of PAB, p-aminohippuric acid (PAH), and their respective acetyl derivatives were carried out on the urines collected for 24 hours after intraperitoneal injection of various amounts of these compounds. Results showed intraconversion among the 4 substances, so that all four appeared in varying proportions after PAB, PAH, or acetyl-PAH were injected. No acetyl-PAH was found after acetyl-PAB was given. The latter observation indicated that conjugation at the carboxyl end was prevented once conjugation had occurred with the amino group. Whenever the extent of acetylation of PAB was diminished by nutritional deficiency or by increasing the dose, conjugation at the carboxyl group was reciprocally increased so that the total proportion conjugated at one position or the other remained constant. The extent of acetylation as affected by pantothenic acid deficiency and by glycine supply (modified by preadministration of benzoic acid or of glycine) has been studied after administrating PAB, PAH, and sulfanilamide.

Availability of panthenol and calcium pantothenate in relation to food intake. S. H. RUBIN, L. DREKTER (by invitation), M. E. MOORE (by invitation) and R. PANKOFF (by invitation).

Nutrition Laboratories, Hoffmann-La Roche Inc., Nutley, N. J. Previous work in this laboratory (*J. Nutrition* 35: 499, 1948) has indicated that the availability of calcium or sodium pantothenate to humans at elevated dosage levels is depressed by food intake to a considerably greater extent than is that of panthenol. Further study of this effect in rats by an excretion technique (*J. Am. Pharm. Assn.* 37: 498, 1948), at dosage levels within the linear working range of 1 to 10 mg/kg. permits the following conclusions to be drawn. *1*) During *ad libitum* feeding, panthenol is approximately 200% (range: 193–267%) as available as equivalent amounts of calcium pantothenate at all doses fed. (A value of 100% denotes equal availability.) *2*) Starvation for 24 hours (postabsorptive state) enhances the absolute urinary excretion of pantothenic acid in response to the 'loading' test doses at all levels, especially after calcium pantothenate. *3*) The relative availability of panthenol and calcium pantothenate in this postabsorptive state is of the order of 220, 140 and 116% at the respective dosage levels of 10, 5 and 2 mg/kg. *4*) In contrast with Silber's results with calcium pantothenate in dogs (*Arch. Biochem.* 7: 329, 1945), the postabsorptive urinary responses are not profoundly affected by the administration of food with the dose. However, a significant increase in fecal pantothenic acid does occur when 10 mg/kg. of calcium pantothenate is given with food, but not with 5 or 2 mg/kg. These results confirm and extend the previous findings in humans that the availability of panthenol is better than that of calcium pantothenate under conditions of higher dosage and *ad libitum* feeding, and is more nearly equal in the postabsorptive state at lower doses.

Vitamin A requirement of the turkey poult fed a purified diet. WALTER C. RUSSELL, M. WIGHT TAYLOR and ROBERT VAN REEN (by invitation). *Dept. of Agricultural Biochemistry, New Jersey Agr. Exp. Sta., Rutgers Univ., New Brunswick, N. J.* Poults fed a purified diet in which protein was supplied as casein and gelatin and vitamin A as the acetate, required at least 3000 IU of the vitamin per kg. of diet (1360 IU/lb.) for normal growth. 5000 IU/kg. of diet (2270 IU/lb.) was required to prevent all external signs of the deficiency, such as ruffled plumage, staggering gait, and cloudiness of the inner eyelid. After 6 weeks on experiment, no liver storage was found even at a level of 6000 IU/kg. of diet (2720 IU/lb.). In all of the groups receiving the purified diet, the lining of the esophagus and crop presented a white furrowed appearance. Histological studies showed the development of very large cells in the epithelial layer with considerable exfoliation. It is not known whether this condition was the result of inadequate vitamin A or due to the physical character of the purified diet. On a practical mash the furrowed condition did not occur. For normal growth and external appearance, 5000 IU of vitamin A per kg. of diet (2270 IU/lb.) are considered adequate.

Metabolism of tryptophan labeled with radioactive carbon. DATTAREYA RAO SANADI (by invitation) and DAVID M. GREENBERG. *Division of Biochemistry, Univ. of California Medical School, Berkeley, Calif.* DL-tryptophan (200 mg.) labeled with C^{14} at the beta-carbon was administered subcutaneously to rats made diabetic with phlorhizin. From the subsequent 24-hour urine collection, glucose was obtained as the phenylglucosazone which was then converted to the phenylglucosotriazole by treatment with cupric sulphate. The ketone bodies were obtained first as the mercury acetone complex and then as 2:4-dinitrophenylhydrazone. The glucose exhibited radioactivity but the acetone was inactive. The activity in the CO_2, obtained by treating the urine with urease, was too low to explain the activity in the glucose as being entirely due to CO_2 fixation. Upon degrading the glucosotriazole by oxidation with sodium periodate it was found that approximately one-fourth of the activity of the glucose molecule was in carbon 6 and another one-fourth in carbons 4 and 5. From the present knowledge of the products of tryptophan metabolism and from the isotopic distribution in glucose it appears that a two carbon unit with C^{14} in the alpha-position is formed from tryptophan and is incorporated in the tricarboxylic acid cycle directly, but this two carbon unit does not form acetoacetate in the diabetic animal. Oxidative deamination and subsequent oxidative decarboxylation of kynurenine would yield the compound $NH_2 \cdot C_6H_4 \cdot CO \cdot C^{14} H_2 \cdot COOH$. In the phlorhizinised animal, this compound may break down to anthranilic acid and a two carbon derivative. The formation of such a derivative has been confirmed by giving a normal rat 10 μc (about 12 mg) of the radioactive tryptophan together with p-amino-benzoic acid. The excreted p-acetylamino-benzoic acid was radioactive.

Vitamin nutriture and tryptophan metabolism in man. HERBERT P. SARETT and GRACE A. GOLDSMITH. *Nutrition Research Laby., Tulane Univ. School of Medicine, New Orleans, La.* Further studies have been conducted on the excretion of nicotinic acid and tryptophan compounds by subjects maintained on a wheat diet, which provides about 40 gm. of protein and 6 mg. of nicotinic acid per day (*J. Biol. Chem.* 167: 293, 1947). The addition of DL-tryptophan to this diet leads to an increase in excretion of N′-methylnicotinamide which is not affected by supplementing the diet with 50 mg. of nicotinamide per day or with a combination of the other B vitamins. The high level of nicotinamide does not decrease the conversion of tryptophan to nicotinic acid compounds. The addition of DL-tryptophan to the diet leads to an increased excretion of two other compounds; one has tryptophan-like activity for *Lactobacillus*

arabinosus, but unlike tryptophan, can be readily extracted from the urine with ether; the other has nicotinic acid-like activity for *Lactobacillus arabinosus* after autoclaving the urine with strong acid. Nicotinic acid or nicotinamide administration does not lead to the excretion of these compounds. The administration of 5 gm. of L-tryptophan per day to 2 subjects with pellagra was followed by some clinical improvement. These subjects excreted much more of the nicotinic acid-hydrolyzable compound than has been obtained after administration of 10 gm. of DL-tryptophan per day to other subjects. The ether extractable tryptophan compound was excreted in smaller amounts by these pellagrins than by subjects receiving the racemic mixture.

Relation of vitamin B_{12} to choline requirement of the rat and chick. A. E. SCHAEFER (by invitation), W. D. SALMON and D. R. STRENGTH (by invitation). *Laby. of Animal Nutrition, Alabama Polytechnic Inst., Auburn, Ala.* A sparing action of vitamin B_{12} on the choline requirement of rats and chicks was noted. The severity and incidence of renal hemorrhage in weanling rats (AES strain) fed diets deficient in choline and substitute nutrients, was markedly decreased by supplementing the diet with a vitamin B_{12} concentrate. Diet 60 (*J. Nutr.* 36: 739, 1948) supplemented with .04% and .06% of choline produced kidney hemorrhage within 14 days with incidences of 100% and 66%, respectively. The addition of a concentrate which supplied 3 μg. of vitamin B_{12} per 100 gm. of diet to diets supplemented with .03%, .04%, and .06% of choline reduced the incidence of renal damage to 50%, 36%, and 0, respectively. Preliminary results indicated that on the basis of methyl equivalents DL-methionine had 100% of the theoretical potency of choline chloride when vitamin B_{12} was added to the diet. Day-old leghorn chicks fed a diet composed of extracted peanut meal 30, extracted casein 6, sucrose 36.9, salts 7, L-cystine .1, cod liver oil 1, lard 19, and synthetic vitamins required .6% of choline for maximum growth in the absence of vitamin B_{12}. Supplementing this diet with 3 μg. of vitamin B_{12} per 100 gm. resulted in an increased growth increment of 75 gm. in 6 weeks. At 8 weeks the weight gain of chicks receiving only .2% of choline plus vitamin B_{12} was equal to that of chicks receiving .6% of choline without vitamin B_{12}.

Iron requirements of 6 adolescent girls. DORETTA SCHLAPHOFF (by invitation) and FRANCES A. JOHNSTON. *N. Y. State College of Home Economics, Cornell Univ., Ithaca, N. Y.* Six healthy girls, 13–14 years of age, who had passed the menarche, were subjects. Iron balance experiments were continued throughout 63 days. Two levels of iron were fed during 2 consecutive 4-week periods which were preceded by a 1-week adjustment period. The iron was provided by basal diets composed of a variety of natural foods. Additional foods, low in iron, were allowed according to choice. Individual retentions were obtained by determining iron in foods, stools, and urines. In Period I, when the mean daily iron intake was 8.6 mg., the retentions were 0.80, 0.86, 1.22, 1.23, 1.42, 1.54, mg/day. In Period II, when the mean daily iron intake was 11.7 mg., the retentions were 0.33, 1.15, 1.54, 1.75, 2.10, 2.22, mg/day. Iron is required by girls of this age for growth and to replace menstrual losses. Menses from each subject were analyzed for 3 or more periods. Mean menstrual losses of iron ranged from 6.12 to 52.57 mg/period. According to the estimations of Heath and Patek (*Medicine* 16: 267, 1937) girls of this age require 0.40 mg. of iron daily for growth. When growth and menstrual losses were considered, 5 of the 6 girls retained enough iron to cover their needs on both levels of intake. Because the menstrual losses of some of these subjects were unusually low, an intake of 8.6 mg. was sufficient. Possibly this intake would not be adequate for many girls of this age.

Distribution and some properties of the mouse salmonellosis resistance factor. HOWARD A. SCHNEIDER. *Laby. of The Rockefeller Inst. for Medical Research, New York, N. Y.* The assay method used to detect the effect of nutrients on the survivorship frequency of mice upon infection with *Salmonella typhimurium* was the Double Strain Inoculation (DSI) test, (*J. Exp. Med.* 87: 103, 1948). The following materials, added to a synthetic diet, have been found to increase survivorship frequencies: whole wheat, corn meal, alfalfa, dried grass juice, brewers yeast, malted barley green and black tea, dried lemon peel. Fractionation studies on whole wheat have shown that activity is concentrated in the germ. White flour is inactive and bran only slightly active. The activity of the germ is diminished on autolysis, but survives dry or wet autoclaving. It is stable over a *p*H range of 1–11. Acetone or chloroform does not extract active material. Active material is extracted from the acetone extracted germ by the use of acid alcohol, but is not extracted with ammoniacal alcohol. Upon transfer into water the activity of acid alcohol extracts is recovered in the water-soluble fraction.

Growth and reproduction of rats on rations devoid of animal protein. M. O. SCHULTZE (introduced by G. M. BRIGGS). *Division of Agricultural Biochemistry, Univ. of Minnesota, Minneapolis, Minn.* A 24% protein ration containing 274.4 gm. soybean sodium proteinate (Archer Daniels Midland Co.); 5.6 gm. DL-methionine; 40 gm. salt mixture; 70 gm. Crisco; 10 gm. corn oil (fortified with 100 mg. dl α-tocopherol, 10,000 IU vitamin A; 1,500 A.O.A.C.U. vitamin D_3); water soluble vitamines (Nelson and Evans, *Arch. Biochem.* 13: 265, 1947), 0.2 mg. folic acid and 598.5 gm. sucrose was fed to weanling rats from females on a ration

containing ethanol extracted casein (55–60°C. for 72 hours) in place of the soybean protein and methionine but otherwise the same components. These rats were bred and their young were fed the soybean ration. On the soybean ration the 6 weeks post weaning weight gains were: for the parent generation, males (12) 172 gm. ± 15.4 (SD); females (26) 123 gm. ± 8.4; for the F_1 generation: males (31) 175 gm. ± 13.6; females (32) 112 gm. ± 12.5, not significantly lower than those on a casein containing ration. To date from 46 pregnancies there were 3 probable resorptions and 43 litters with 14 dead and 263 live young. Of the latter 14 whole litters and 101 young failed to survive the third day after birth. Of 162 rats surviving the third day, 153 (94.4%) were weaned at 21 days with a mean weight of 35.6 gm. The cause of the high early mortality is not known but in no case could it be ascribed to failure of the young to obtain sufficient milk since lactation performance in all cases was excellent.

Nutrition of women during normal and abnormal pregnancy in Panama and the Canal Zone. NEVIN S. SCRIMSHAW (by invitation), MARTHA J. THOMASON (by invitation), ROBERT P. BAYS (by invitation) and ESTELLE E. HAWLEY. *Dept. of Obstetrics and Gynecology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* The dietary intake of 308 pregnant women representing 5 distinct economic or racial groups in Panama and the Canal Zone was studied by diet interviews. The incidence of pre-eclampsia for each of these groups in over 12,000 deliveries did not indicate a true tendency for this condition to occur more frequently in groups with a lower average nutritional intake. Furthermore, when the diet histories of 75 women with pre-eclampsia were analyzed, there was no reasonable statistical possibility that their diets were poorer than those of their own racial or economic group in general. Blood samples from 242 pregnant women representing the above 5 groups and 2 interior villages were analyzed for total protein, vitamin A, carotene, free and total riboflavin and alkaline phosphatase. Alkaline phosphatase showed little variation with diet and economic status, increased with gestation, and appeared to be lower in the white Americans than in the groups with greater skin pigmentation. Of the 5 groups studied in detail, the one with the lowest estimated protein intake had significantly higher serum total protein values than did the others. Women in the villages of La Gartarita and Parita had serum total protein values considerably above those reported for the United States or the Canal Zone, despite a lower protein intake. However, the women from these villages average much lower in serum carotene values. These and other biochemical data are discussed in relation to the diet histories and the clinical states represented.

Vitamin C deficiency in the ringtail monkey. JAMES H. SHAW. *School of Dental Medicine, Harvard Univ., Boston, Mass.* The ringtail or brown capuchin monkey (*Cebus fatuellus*) is of interest in dental investigations because of its relatively small adult size and its tractability. Investigations of the ringtail monkey's need for various nutrients have been conducted in this laboratory to determine how similar its nutritional requirements were to those of man and the rhesus monkey. In a study of whether vitamin C was required in the diet, 10 young adult ringtails were offered a purified diet which was believed to be adequate in all known nutritional essentials but deficient in ascorbic acid. These monkeys maintained their original body weight, normal appearance and activity reasonably well for periods of 12 to 20 weeks when the classical manifestations of a chronic state of scurvy began to appear. The deficiency was characterized by rapidly increasing debility, moderate decreases in body weight, extreme tenderness of the joints and extensive hemorrhages over large areas of the head, arms and legs. Oral lesions were particularly severe and rapidly progressing, with widespread hyperemia, hypertrophy and necrosis of the gums accompanied by extensive destruction of alveolar bone and sloughing of the teeth. The injection or oral administration of crystalline ascorbic acid soon after gross manifestations of scurvy appeared resulted in extremely slow but complete recovery. Administration of ascorbic acid at later stages was ineffective. Comparable ringtail monkeys offered the same diet supplemented with adequate ascorbic acid were maintained in normal health throughout the experimental period.

Nutritional evaluation of the iso-oleic acids: Elaidic, petroselinic and petroselinelaidic acids. HENRY SHERMAN (by invitation), LAURA M. CAMPLING (by invitation), WILLY LANGE (by invitation) and ROBERT S. HARRIS. *Nutritional Biochemistry Lab., Massachusetts Inst. of Technology, Cambridge, Mass.* Male weanling rats were fed diets consisting of 20% casein (Labco) 61.5% corn starch, 14% fat, 4% salts and liberal quantities of 14 vitamins. The fat in each of four diets varied as follows: olive oil randomized with an equal quantity of *a*) trielaidin ($\Delta^{9:10}$ trans), *b*) tripetroselinin ($\Delta^{6:7}$ cis), *c*) tripetroselinelaidin ($\Delta^{6:7}$ trans) or *d*) pure olive oil. Olive oil was used because it contains 80-85% $\Delta^{9:10}$ cis oleic acid. Distilled water and diets were supplied *ad libitum* for 42 days and rats were weighed biweekly. Before sacrifice data were taken for body surface calculations. The fatty acids extracted from the various groups of pooled carcasses were analyzed for iodine value, neutralization value and arachidonic acid content. The results indicate that rats on *a* grew less rapidly, had smaller body surface and the carcass fat contained less arachidonic acid than rats fed *d*. Rats fed *b* grew better than those fed *c* and their carcass fat contained more arachidonic acid. Rats fed *c* grew less and de-

posited less arachidonic acid than those fed *d*. Rats fed *b* grew as well as those fed *d*, but their carcass fat contained less arachidonic acid. Cis isomers of $\Delta^{6:7}$ and $\Delta^{9:10}$ oleic acids appear to be more nutritious than their respective trans isomers, and the $\Delta^{6:7}$ trans isomer more nutritious than the $\Delta^{9:10}$ trans isomer.

Influence of dietary protein and fat on the toxicities of trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT) for the rat. MAURICE E. SHILS and LEONARD J. GOLDWATER (introduced by C. G. KING). *Division of Industrial Hygiene, School of Public Health, Columbia Univ., New York, N. Y.* Conflicting reports have been published on the effect of diet on the closely related compounds TNT and DNT. A study was made of the comparative effects of protein, fat and carbohydrate on both TNT and DNT toxicity by feeding to rats diets which contained low and high levels of each of these nutrients. The intake of each toxic compound was kept equal for each group. The growth inhibiting effect of DNT (fed initially at 300 mg. % level in low fat diets and 600 mg. % level in high fat diets) was less pronounced when the fat content of the diet was increased from 6.0% of the dietary calories to 60.0%. The beneficial effect of fat was noted on both the high and low protein diets. Variations in fat intake had little effect on the growth of rats on low protein diets receiving TNT (fed initially at 150 mg. % level in low fat diets and 300 mg. % level in high fat diets). The DNT and TNT-treated rats and their controls on the diets containing protein equivalent to 25% of the calories all grew at better rates than similarly treated animals on diets containing protein at half this level. The treated groups grew less than the controls but since the absolute increases in weight of each of these three groups in response to the higher protein intake was of the same order of magnitude, it appears that protein has little or no specific effect in overcoming the growth inhibition of the toxic compounds. However, at the higher protein intake there were no deaths in the DNT-poisoned rats as compared to a high mortality on the low protein diets.

Radioactive phosphorus in the study of caries-resistant and susceptible albino rats. RAY L. SHIRLEY (by invitation) and CARL A. HOPPERT. *Kedzie Chemical Laboratory, Michigan State College, East Lansing, Mich.* In 1931–32 Hoppert et al. demonstrated that dental caries could be developed in rats by feeding diets containing coarsely ground corn or rice. Subsequently, a genetic study was undertaken with Hunt in which two distinct strains of Albino rats were developed that vary markedly in their susceptibility to dental caries. The present study was undertaken to compare some chemical constituents of the teeth of these developed strains. The caries resistant and susceptible rats have been demonstrated in this investigation not to differ significantly in the following respects: *a*) weight of teeth; *b*) % ash; *c*) % phosphorus on the basis of dry weight, and in the ash; *d*) rates of deposition and removal of radioactive phosphorus in the teeth when the isotope was injected into the peritoneal cavity; *e*) occurrence of radioactive phosphorus in the teeth of offspring of females that were injected intraperitoneally with the isotope during pregnancy; *f*) the in vitro adsorption of the isotope by the teeth from aqueous solution. The data obtained indicates that the differences in susceptibility to dental caries of the rats used in this study are not due to differences in the phosphorus metabolism of the teeth.

Determination of vitamin-D activity in the rat by means of radioactive phosphorus. R. H. SNYDER (by invitation), HAROLD J. EISNER (by invitation) and HARRY STEENBOCK. *Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wis.* In the course of an investigation of the mechanism of vitamin-D activity it was found that the response of the rachitic rat to the administration of radiophosphorus could be directly quantitated to small amounts of vitamin D. The vitamin was administered orally in a single dose a short time before the tracer was given intraperitoneally. An external 'count' of the forepaw of the anesthetized rat was chosen as the simplest technique of measurement. A relatively simple apparatus was designed for placing a rat in a reproducible position under a mica end-window G. M. tube for counting. Though a difference in the external count due to D developed almost immediately after administration of the P^{32} it was found that the best time to take the counts was from 8 to 10 days later. The counts of groups with different doses were compared with a control group which received no vitamin D, or with a group receiving a known dose, and/or with previously established reference curves. Although no attempt was made to develop the method exhaustively, the collected data indicate quite definitely that it is possible to determine vitamin D accurately over a range of from one-half to 40 units with groups of 10 animals each. Good results were obtained using a preparatory period as short as 14 days on ration 2965.

An unidentified dietary factor concerned in human growth. SELMA E. SNYDERMAN (by invitation), ROSA LEE NEMIR (by invitation) and L. EMMETT HOLT, JR. *Dept. of Pediatrics, New York Univ. and the Children's Medical Service, Bellevue Hospital, New York City.* In the course of studies designed to measure the requirements for B vitamins we have had occasion to feed a number of infants on purified diets. The diets were constructed from vitamin-free casein, crisco and a special devitaminized dextrimaltose; they provided 15% of the calories as protein, 35% as fat and 50% as carbohydrate. This was supplemented by a mineral mixture, a fish oil concentrate to supply fat soluble vitamins and a mixture of water soluble vitamins containing ascorbic acid, thiamine, riboflavin, nico-

tinamide, pyridoxine, pantothenic acid, inositol, choline and p-amino benzoic acid. Biotin and pteroyl glutamic acid were added in more recent studies. Only one component of the vitamin mixture, that which was being studied, was varied at a time. Barring a few intercurrent infections, the infants remained in good health and gained weight normally. It was observed, however, that if the experimental diet was continued for more than 5 to 6 months a cessation of weight gain would occur regularly, although the infants still gave every appearance of health. Attempts to supplement the experimental diet with amino acids or by increasing the quantities of B vitamins failed to restore weight gain. The addition of Brewer's yeast caused resumption of weight gain in from 5 to 23 days, but the weight gain was in some instances subnormal. Liver preparations have given inconclusive results. Return to natural food caused resumption of normal weight gain at once.

Influence of dietary proteins on the urinary excretion of amino acids by human subjects. BETTY F. STEELE (by invitation), MAY S. REYNOLDS (by invitation) and C. A. BAUMANN. *Dept. of Biochemistry and Home Economics, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* Two separate controlled diet studies were made. In the first study 3 adults ingested diets containing 25, 50 and 100 gm. of protein/day for periods of 6 days. The principal sources of protein in this study were eggs and cottage cheese. In the second experiment 4 subjects consumed diets containing 25, 100 and 200 gm. of protein/day for 6 days. The main sources of protein were ground beef, white bread and milk. Twenty-four hour samples of urine were collected and analyzed microbiologically for 18 amino acids. Both 'free' and 'total' amino acids were determined in the urines, and the amino acid contents of the diets ingested were also determined. Large amounts of 'free' histidine and cystine were present in the urine samples. Glutamic acid, glycine, histidine, aspartic acid, serine, proline, threonine, tryptophan and methionine were present in fairly large quantities in samples of hydrolyzed urine. Increasing the protein intake four fold caused roughly a 2-fold increase in the excretion of alpha amino nitrogen. Of those amino acids present in large amounts, histidine and threonine excretion appeared to bear a positive relationship to intake. Glutamic acid, aspartic acid, tryptophan and methionine excretion seemed to be independent of the amount of the amino acid ingested. Many of the other amino acids were present in urine in only low concentrations.

Ascorbic acid metabolism of older adolescents. CLARA A. STORVICK, BESSIE L. DAVEY (by invitation), RUTH M. NITCHALS (by invitation), RUTH E. COFFEY (by invitation) and MARGARET L. FINCKE. *Nutrition Research Laboratory, Agr. Exp. Sta., Oregon State College, Corvallis, Ore.* In 1946–47, 4 levels of ascorbic acid intake were studied during periods of 7 days each, and in 1947–48, 3 levels were studied during periods of 10 days each. The subjects, 8 girls and 8 boys, were 16 to 19 year-old Freshmen. During the first year, 1946–47, the subjects received the following for periods of 7 days each: *1*) their usual diets; *2*) a daily supplement of 200 mg. of crystalline ascorbic acid in addition to the ascorbic acid in the food; *3*) the total daily ascorbic acid intake as recommended by the National Research Council; and *4*) 10 mg. of ascorbic acid less than they received during the third period. During the second year, 1947–48, the subjects received the following for periods of 10 days each: *1*) a daily supplement of 200 mg. of crystalline ascorbic acid in addition to the ascorbic acid in the food; *2*) the total daily ascorbic acid intake as recommended by the National Research Council; and *3*) 10 mg. less than they received during the second period. The recommended allowance of the National Research Council did not maintain mean plasma values as high as their respective means during the saturation period when they received 200 mg. supplements of crystalline ascorbic acid. A decrease in ascorbic acid intake to 10 mg. less than the recommended allowance resulted in a statistically significant decrease in plasma ascorbic acid concentration for only 2 of the 8 girls and for 1 of the 7 boys (values for one boy were not included because of illness).

Role of pyridoxine in economy of food utilization. BARNETT SURE and LESLIE EASTERLING (by invitation). *Univ. of Arkansas, Fayetteville, Ark.* Twenty four male and 24 female albino rats were depleted of pyridoxine. In 26 to 59 days 15 pairs developed dermatitis which were used for this study which was conducted in pairs. In each pair the positive control, which received 25 μg. pyridoxine daily, was allowed the same amount of food consumed by its litter mate, which received no pyridoxine. The average results were as follows,— P representing the pathological or pyridoxine-deficient animals, and C the litter mate controls which received pyridoxine. Food consumption: P, 325 gm., C, 317 gm.; gains in body weight: P, 37.5 gm., C, 77.3 gm.; gains in weight/100 gm. food intake; P, 11.4 gm., C, 25.2 gm.; changes in chemical composition of body gains: fat: P, −0.24 gm., C, +8.1 gm.; protein: P, 9.3 gm., C, 17.5 gm.; ash: P, 2.2 gm., C, 3.4 gm.

Synthesis of amino acids in the rumen. W. E. THOMAS (by invitation), J. K. LOOSLI, FENT H. FERRIS (by invitation), HAROLD H. WILLIAMS and L. A. MAYNARD. *Depts. of Animal Husbandry and Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.* Growing goats and sheep were fed purified diets that contained, at a 10% protein-equivalent level: *1*) casein; *2*) casein hydrolysate plus trypto-

phan; and *3*) urea as the only source of nitrogen. Normal growth was obtained with diets *1*) and *2*) and ¾ normal with diet *3*). Biological values, determined from nitrogen balance studies, were higher for diets *1*) and *2*) than for *3*). The content of 10 amino acids (those that have been shown to be essential for rats) of the feeds and of rumen and excreta samples was determined microbiologically. The results showed that the essential amino acid content of the rumen material was similar for the urea and casein diets. Although traces of amino acids were found in the purified diet where urea was given as the sole source of nitrogen, the amounts in the rumen per gram of total nitrogen were 9 to 20 times that ingested. Furthermore the daily loss of each of the essential amino acids in the excreta more than accounted for the traces found in the purified diet. These data together with the surprising growth of the animals indicated a remarkable synthesis of amino acids from urea nitrogen by rumen organisms.

Nutrition of L. Bifidus, predominant intestinal organism of the breast fed infant. R. M. Tomarelli (by invitation), R. F. Norris (by invitation), Paul Gyorgy. *Univ. of Pennsylvania Hospital, Philadelphia*; J. B. Hassinen (by invitation) and F. W. Bernhart (by invitation), *Wyeth, Inc., Mason, Mich.* In the isolation of strains of bifidus anaerobically from breast fed infant stools it was observed that better growth was had if milk were added to the conventional liver infusion medium. Skimming did not decrease activity. An ether soluble fraction was isolated whose activity could be replaced by Tween 80, methyl oleate, oleic, linoleic or vaccenic acid. The exact nature of the factor in milk is unknown at present. The various strains of bifidus could be grown on a synthetic medium containing acetate buffer if milk or oleate were added. Human milk contains approximately 4 times as much activity as cow's milk. With citrate as the buffer, milk, but not oleate, yielded growth. Citrate proved toxic to the organism, the toxicity being neutralized by the addition of Ca of the milk. The strains varied in their sensitivity to citrate toxicity. Citrate toxicity is the result of the binding of essential minerals. Mn appears to be the only mineral affected, since removal of Mn but not of Fe nor Mg from the acetate medium decreased growth. The utilization of glucose and especially of lactose was influenced by the autoclaving of the carbohydrate with the medium. Unautoclaved lactose was not utilized even after 64 hour incubation. When sterile milk was used as a source of unautoclaved lactose, growth was had in 40 hours indicating the presence of a factor in milk that rendered lactose available. Several strains of bifidus were isolated that required unknown growth factors in enzymatic digest of casein.

Relation of factor in a methanol extract of liver and pteroylglutamic acid in mink. S. B. Tove (by invitation) and C. A. Elvehjem. *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison, Wis.* The previously reported (*Proc. Soc. Exp. Biol. & Med.* 62: 169, 1946) pteroylglutamic acid (PGA) deficiency syndrome in mink can be relieved by the administration of a methanol extract of fresh liver (fed at a level of 1 ml/100 gm. ration) which contains only 2γ PGA/ml. If this level of extract is added to the ration from the start, symptoms of a PGA deficiency fail to occur. The addition of 0.3% of a crude antagonist (7-methyl PGA) to a diet containing 0.1 mg. PGA/100 gm. ration resulted in rapid loss of body weight and hemoglobin. When the inhibitor is added to a ration containing the extract, no deficiency syndromes were seen. Microbiological assay of the PGA content of the blood, urine and feces of mink receiving 0.0, 0.025, 0.1 mg. PGA/100 gm. ration, and the extract in addition to the low and high levels of PGA indicate that the extract caused an increase in the PGA greater than can be accounted for by the PGA in the extract. Mink receiving the basal diet or this diet supplemented with PGA fail to respond when given vitamin B_{12} preparations or commercial liver extracts used in pernicious anemia therapy, but when the methanol extract is given a response is obtained. It is thus apparent that the methanol extract factor, which is different from vitamin B_{12}, has a sparing action on pteroylglutamic acid.

Tyrosine excretion in rheumatoid arthritics and normals. E. B. Wallraff (by invitation), C. A. Stephens (by invitation), A. Borden (by invitation), P. Holbrook (by invitation), D. Hill (by invitation), L. J. Kent (by invitation) and A. R. Kemmerer. *Dept. of Nutrition, Univ. of Arizona, and Southwestern Clinic and Research Institute, Tucson, Ariz.* Increased tyrosine excretion in pregnancy has been reported (Lawrie, *Biochemical J.* 41: 41, 1947). The relief of the rheumatoid arthritic syndrome during pregnancy has occasioned investigations to determine the possible mechanism of relief. Using a slight modification of the microbiological procedure for urines of Woodson et al (*J. Biol. Chem.* 172: 613, 1948) and the medium of Henderson and Snell (*J. Biol. Chem.* 172: 15, 1948), tyrosine was assayed in 24-hr. urine specimens from rheumatoid arthritics and normals as well as pregnant rheumatoid arthritics and pregnant normals. It appears that tyrosine which is bound so as to be microbiologically unavailable without hydrolysis is higher in rheumatoid arthritic urine than in normal urine. Diet analysis for caloric and protein intake indicates no significant differences between the two groups. The difference may in part be due to differences in assimilation of protein, or may possibly be due to metabolic abnormality. Studies on excretion of other amino acids are in progress.

THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Thirty-third Annual Meeting

DETROIT, MICHIGAN, April 18–22, 1949

(For possible corrections in any of the following abstracts see the June issue)

Studies on the enzymatic hydrolysis of the capsular polysaccharide of Klebsiella pneumoniae Type B. EDGAR E. BAKER (by invitation) and WALTHER F. GOEBEL. *Laboratories of The Rockefeller Institute for Medical Research, New York City.* It has been observed that the capsular polysaccharide of *Kl. pneumoniae* Type B (FrB), when administered to embryonated eggs is capable of inhibiting the multiplication of mumps virus. In order to obtain degradation products of FrB which could be used for studying the mechanism of this inhibitory action, a search has been made for a specific enzyme. A motile, gram-negative, spore-forming bacillus has been isolated from soil, which elaborates an enzyme capable of hydrolyzing the polysaccharide. Although the microorganism will grow in a medium containing di- or trisaccharides, but not monosaccharides, it can elaborate the enzyme only when FrB is present. Ammonium or nitrate ions, casamino acids or peptone may serve as sources of nitrogen. Growth factors are not required. The enzyme may be obtained from culture filtrates of the microorganism grown in the presence of 0.1% FrB. It may be prepared more economically, however, by first culturing the bacilli in a medium containing sucrose and then resuspending the organism in a small volume of medium containing FrB. The enzyme specifically hydrolyzes solutions of FrB. The former can be determined quantitively by observing the rate of change of viscosity at 30° and at *p*H 5.0. The drop in viscosity of the polysaccharide is followed by a loss of serologic activity. If the hydrolysis proceeds to completion, the liberation of reducing sugars occurs. It is not yet known whether the depolymerization of the carbohydrate and its hydrolysis are caused by a single enzyme.

Comparison of methods of determining differences in virulence for mice of strains of salmonella typhosa. H. C. BATSON, MAURICE LANDY and MARTHA BROWN (introduced by MARION C. MORRIS). *Dept. of Biologic Products, Army Medical Dept. Research and Graduate School, Army Medical Center, Washington, D. C.* Methods employed for determining the virulence for mice of strains of *S. typhosa* have been of two main types. The first, as utilized almost exclusively by British workers, is based on the intraperitoneal injection of graded doses of the organisms suspended in saline. The second, as employed by most American workers during the past decade, is essentially similar but incorporates the use of 5% hog gastric mucin as a suspending medium. A third method which has shown some promise is based on intracerebral injection without the aid of an adjuvant. A direct comparison of the efficacy of these methods in detecting differences in virulence of strains of *S. typhosa* previously has not been made. Such a comparison was the objective of this investigation. Strains of *S. typhosa* grown under different but defined nutritional conditions were tested for their virulence for mice by the 3 methods described. Differences in virulence of the strains could not be determined satisfactorily by the intracerebral method. Fatalities resulting from different strains of organisms were comparable but in each case were only poorly related to the size of challenge dose employed. Differences between the strains were more distinct when the organisms were administered intraperitoneally as saline suspensions. Furthermore, mortality was more clearly dependent upon the size of the challenge dose. With the use of mucin as an adjuvant, differences in virulence of strains of organisms were amplified markedly and became readily apparent. The effect of dosage on mortality was greatly accentuated. It appears that assessment of the virulence of strains is dependent upon the method of testing employed. The method incorporating the use of mucin as an adjuvant was the most sensitive in detecting differences in virulence; the intracerebral method was the least sensitive.

Further study of toxins and antigens of *Shigella dysenteriae*. DANIEL A. BOROFF and BEATRICE P. MACRI (by invitation). *Camp Detrick, Frederick, Md., Dept. of Bacteriology, and George Washington Univ., Washington, D. C.* In previous studies of serological and immunological properties of toxins and antigens of *Shigella dysenteriae* (Boroff, D. A., Federation Proc. 7: 301, 1948) a suggestion was offered that there may be only 1 dominant antigen in *S. dysenteriae*. In these studies the data obtained in active protection experiments indicate that it is possible to immunize rabbits with either smooth

or rough whole organisms of *Shigella dysenteriae* or with their respective toxins and thus induce active protection against lethal doses of these substances. Not only whole organisms or solutions of whole organisms afford protection against each other but both chemically and physically purified toxins protected the animals and induced the formation of identical and reciprocally absorbable antibodies. This phenomenon of cross protection is not in accord with the concept that 2 toxins and 2 distinct antigens exist in *S. dysenteriae*. It is, however, explainable by the presence of 1 dominant antigen in the organism. Furthermore, the protection afforded rabbits against the whole organisms of the smooth variant by immunization with toxic lysates of the rough variant lends additional support to the latter hypothesis.

Immunologic relations and comparative invasiveness of strains of poliomyelitis from recent epidemics. GORDON C. BROWN (by invitation), THOMAS FRANCIS, JR. and JOHN D. AINSLIE (by invitation). *Dept. of Epidemiology, School of Public Health, Univ. of Michigan, Ann Arbor, Mich.* The immunologic relationship of strains of poliomyelitis virus was determined by means of virus neutralization tests performed in monkeys. The strains of virus had been obtained from the following epidemics: Cleveland, 1941; Texas, 1943; Buffalo, 1944; Rockford, Illinois, 1945; Tennessee, 1945; Ann Arbor, 1945, and Minnesota, 1946. Several dilutions of each virus were mixed with serum obtained from monkeys convalescent to infection with the various strains under study. Complete reciprocal testing between strains has not been possible but the results clearly demonstrate that all the viruses, with the exception of the Tennessee strain, are closely related immunologically. While studying each of these strains of virus certain characteristic differences in invasiveness were noted. After compiling data from all monkeys infected with each strain it was observed that variation occurred in the percentage showing fever, the height and duration of fever, the percentage, type and degree of paralysis and other clinical signs. Certain of these variations were so characteristic as to partially identify the strain of virus.

Evidence for the controlling influence of protease-inhibitors in allergic phenomena. KENNETH L. BURDON. *Dept. of Bacteriology and Immunology, Baylor Univ. College of Medicine, Houston, Texas.* Traditional theories about allergy, based entirely upon the amount and distribution of antibodies, and upon the supposedly crucial importance of the site of antigen-antibody union in the body, have never been adequate to explain satisfactorily the diverse manifestations of hypersensitivity in man and in different animal species. Yet some universal, underlying mechanism must operate. There is increasing evidence that such a mechanism is to be found in the system of natural proteases, their inhibitors and activators, present in body fluids. According to this concept, allergic reactions are initiated by the sudden activation of natural proteases, following antigen-antibody combination, and are prevented by the action of protease-inhibitors. New support for this view has been obtained by use on an extended scale of a greatly improved procedure permitting the strictly quantitative titration of the antitrypsin in samples of blood serum and other fluids by a rapid, routine test. Results with several hundred specimens from allergic persons and normal controls, and from sensitized guinea pigs, have shown a sudden drop in antitrypsin in the first seconds of anaphylactic shock, a rise following sub-lethal reactions, and a consistent association of an especially high tryptase-inhibitor titer with the 'desensitized' state, whether this condition was induced by specific or by non-specific means. It was demonstrated that the 'anergy' to tuberculin exhibited at times by some tuberculous men and animals is always accompanied by a markedly increased antitryptic power of the blood.

Prophylactic immunity to experimental enteric cholera. WILLIAM BURROWS and LAWRENCE WARE (by invitation). *Dept. of Bacteriology and Parasitology, Univ. of Chicago, Chicago, Ill.* It was shown earlier that experimental enteric cholera in the guinea pig produced by overwhelming doses (viz., 20 mg.) of the vibrios is sharply modified by prior active immunization; in the immunized animal the percentage vibrios of the total fecal flora is reduced from 50–70% to 1–10% and the period of excretion shortened from 2–3 weeks to 5–7 days. If the inoculum is reduced both of these effects are observed in the normal animal but the ratio of total vibrios excreted to those inoculated becomes very large, many thousand fold, indicating that a true infection is produced. With progressive reduction a minimal dose infecting 50% of the animals (ID_{50}) may be calculated by the usual methods, in this case by inverse probability. The ID_{50} dose for the normal 300–400 gm. guinea pig has been found to be 0.08 mg. (32 million vibrios). In animals immunized with 0 vaccine and infected 4 days after the last of 3 inoculations, the ID_{50} is raised to 1.13 mg., a 14.1 fold increase over the normal. Two weeks later the immunity has fallen off so that the ID_{50} is 0.170 mg., an 8.7 fold increase over the normal. Following passive oral immunization the ID_{50} is immediately raised to 0.36 mg., a 4.05 fold increase, and rises to 0.51 mg., or a 6.4 fold increase 2 weeks later, the latter coinciding with maximum excretion of absorbed antibody. It is concluded that the presence of antibody in the feces is associated with prophylactic immunity to experimental enteric cholera in the guinea pig.

Purification of antibodies by specific methods. DAN H. CAMPBELL and LEONARD LERMAN (by invitation). *Gates and Crellin Laby. of Chemistry, California Inst. of Technology, Pasadena, Calif.* Methods have been developed for the efficient removal and purification of antibodies from serums. These methods are based on the removal of antibody with soluble or insoluble antigens and subsequent dissociation with simple specific hapten or dilute acid or both. Recent experiments with adsorption columns or suspensions of R-azo-coagulated egg white and R-azo-phenol-formaldehyde resins indicate that such systems usually remove more antibody than is realized by use of soluble precipitating antigens. Studies have been made chiefly with rabbit antibodies against ovalbumin and p-azophenyl arsonate, and include quantitative studies on specific adsorption.

Interference with induction of the anaphylactic state by prior feeding of a hapten-like allergenic chemical. MERRILL W. CHASE. *Rockefeller Inst. for Medical Research, New York City.* The feeding of allergenic chemicals, such as 2:4 dinitrochlorobenzene and picryl chloride to guinea pigs that have not previously encountered the respective substance, conditions the animals so that the outcome of subsequent attempts to induce hypersensitivity is profoundly modified. The conditioned animals fail to respond, or they respond abortively, to various sensitizing procedures effective in normal animals. This inhibitory effect is observed not only when attempts are made to develop a specific dermal sensitivity of the 'delayed type' but also when anaphylactic sensitization is undertaken. Guinea pigs that have been fed picryl chloride, for example, and have then been treated with an 'artificial antigen' made by coupling the proteins of guinea pig serum with the picryl radical as the serologically determinant grouping show no or scarcely any anaphylactic effects upon challenge with other conjugates having the same determinant grouping. There exists, accordingly, a specific mechanism that can modify the outcome of sensitizing procedures and that results in a certain level of tolerance; the influence of this mechanism is probably encountered also in natural exposures of human beings to sensitizing agents.

Comparative response and persistence of immunity in mice vaccinated intra-abdominally or by gavage with *Shigella sonnei*. MERLIN L. COOPER, HELEN M. KELLER and AGNES M. HART (introduced by ALBERT B. SABIN). *Children's Hospital Research Foundation and Dept. of Pediatrics, College of Medicine, Univ. of Cincinnati, Cincinnati, Ohio.* One group of mice was vaccinated intra-abdominally with one injection of 100 million killed *Shigella sonnei* as broth vaccine. A control group was given intra-abdominally 1 injection of an equal volume of uninoculated broth medium. Another group of mice was given by gavage, daily for 15 days, one billion killed *Sh. sonnei* as broth vaccine. A control group was given by gavage, daily for 15 days, an equal volume of uninoculated broth medium. One week, 6 weeks and 12 weeks following completion of vaccination, mice from the 2 vaccinated groups and the 2 control groups were challenged intra-abdominally with the homologous *Sh. sonnei* suspended in 3% sterile mucin. The mice vaccinated intra-abdominally withstood after 1 week more than 24,000,000 LD_{50} doses of culture, after 6 weeks 5,000,000 LD_{50} doses and after 12 weeks 1,700,000 LD_{50} doses. The mice vaccinated by gavage withstood after 1 week 263,000 LD_{50} doses, after 6 weeks 5,600 LD_{50} doses and after 12 weeks 85 LD_{50} doses. The range of LD_{50} doses of culture withstood by the groups of control mice was from less than 1.0 to 3.0. Data will also be presented regarding the passive mouse protective powers and agglutinative titers of sera obtained from each group of mice 1, 6 and 12 weeks following completion of vaccination.

Antisheep agglutinins in infectious mononucleosis; further studies. I. DAVIDSOHN and CHIYO KASHIWAGI (by invitation). *Mount Sinai Medical Research Foundation and Chicago Medical School, Chicago, Ill.* In over 200 patients with infectious mononucleosis antisheep agglutinins were studied in the serum. Earlier observations on the ability of certain antigens to remove the agglutinins were confirmed: beef erythrocytes completely absorbed the agglutinins present in infectious mononucleosis, whereas guinea pig kidney was unable to do so. In more than ⅔ of the tests, the guinea pig antigen removed from 50–75% of the originally present agglutinins; less than $\frac{1}{10}$ of the tests showed a maximum absorption of 87.5%; and in no instance of infectious mononucleosis was complete removal of the agglutinins observed. According to recent reports bovine albumin as diluent raises the titer of antisheep agglutinins in infectious mononucleosis. This problem was reinvestigated by studying the effect of several diluents on the titers of the agglutinins. Under standard conditions, bovine albumin and human plasma used instead of saline as diluent lowered the titers in both the absorbed and unabsorbed serums, or left them unchanged. Investigation of the effect of time and temperature of incubation showed that prolonged incubation at ice-box temperature increased the titers, and hence the sensitivity of the test. This effect was independent of the diluent. Studies are also reported on the preparation of the guinea pig kidney and beef red cell antigens used in the differential test.

Polysaccharides isolated from tissues of normal, immune, and immunologically paralyzed mice. LLOYD D. FELTON, BENJAMIN PRESCOTT (by invitation), GLADYS KAUFFMANN (by invita-

tion) and BARBARA OTTINGER (by invitation). *Laby. of Infectious Diseases, Microbiological Inst., National Inst. of Health, Bethesda, Md.* A preliminary observation has been reported that, when mice were inoculated with a large dose (0.5 mg.) of pneumococcus antigenic polysaccharide, their immunological system was 'paralyzed' so that they failed to respond to a subsequent immunizing dose. From the tissues of such mice it was possible to separate a soluble antigen which was antigenic in both precipitin and active immunity tests. In continuing this study, a comparison has been made of the polysaccharide which has been obtained from the tissues of a) normal mice of C_3H strain, b) mice immunized with 0.002 mg. of antigenic polysaccharide of each type, I, II, and III respectively, and c) mice immunologically paralyzed with type I, II, and III. The tissues studied included: blod, heart, lung, liver, kidney, spleen, lymph, thymus, stomach, bone marrow, muscle, and skin. Like tissues from 10 mice of each of these groups were pooled, ground, and a polysaccharide obtained by methods employed for isolating antigenic polysaccharide from pneumococci. The product, a dry white powder, was soluble in 7.5% trichloracetic acid and at neutral *p*H, and insoluble in alcohol. In all alike; tests for glycogen and protein were negative. The polysaccharide so obtained from tissues of normal mice failed to demonstrate antigenic activity. The immunized mice gave low yields of polysaccharide which showed a comparatively low titer immunity in 9 of the 12 tissues of the type I mice, in 10 of the type II tissues, and in 6 of the type III tissues. The immunologically paralyzed mice, on the other hand, yielded greater amounts of polysaccharide. Furthermore, the immunizing titer was appreciably higher in the polysaccharides from the tissues of these 'paralyzed' mice.

Procaine penicillin and sulfonamide antagonism. H. FISHBACK (by invitation), H. WELCH, E. Q. KING (by invitation), J. LEVINE (by invitation), C. W. PRICE and W. A. RANDALL. *Federal Security Agency, Food and Drug Administration, Washington, D. C.* Procaine has long been known as an antagonist of the sulfonamides. This antagonism may result through direct action of the procaine as such or through conversion of the procaine to p-aminobenzoic acid. The accepted ratio of procaine-antisulfonamide action is much lower than the PABA-antisulfonamide action. The introduction of procaine-penicillin as a chemotherapeutic agent raised the question as to whether sufficient procaine or procaine derivatives would be formed in the body to act antagonistically toward sulfonamides during combined therapy. Accordingly, 1,500,000 units of procaine penicillin for aqueous injection was administered to 37 individuals and blood was drawn for examination 3 hours after the injection. The penicillin levels were determined and the serum examined for procaine, p-aminobenzoic acid and an acylated derivative of p-aminobenzoic acid. The concentration of penicillin varied from 0.5–8.0 U/ml., averaging 2.5 U/ml. The blood concentration of procaine was approximately 0.1 μg/ml. in 68% of the cases. None could be detected in the remainder. A level of 0.1 μg/ml., for PABA was found in only 18% of the cases. However, previous reports indicate that this concentration of PABA is sufficient to antagonize the therapeutic action of sulfanilamide but insufficient to interfere significantly with the therapeutic action of sulfathiazole or sulfadiazine. An acylated derivative of PABA was found in each of the 37 individuals in quantities ranging from 0.2–1.0 μg/ml. *In vivo* experiments are now under way to determine the actual antagonism when experimental infections are treated with combined therapy.

Neutralization technique in *Tsutsugamushi* disease (scrub typhus) and the antigenic differentiation of Rickettsial strains. JOHN P. FOX (introduced by P. K. OLITSKY). *International Health Board, Rockefeller Foundation, New York City.* Neutralization of *R. orientalis* by homologous antiserum was most effectively demonstrated when mice were inoculated by the intraperitoneal route with mixtures of undiluted antiserum and serial dilutions of infective material. Satisfactory results could not be obtained by employing a constant infective dose and serial dilutions of antiserum nor by using the subcutaneous or intracerebral routes of inoculation. Furthermore, neutralization was independent of complement. Evidence was obtained to suggest that neutralization depends principally upon a union of antigen and antibody *in vitro*; however, passive immunity may best explain the zone phenomenon often observed. The satisfactory expression of the neutralizing capacity of an antiserum was rendered difficult by the above mentioned zone phenomenon and by the otherwise frequently irregular pattern of mouse survival observed in neutralization tests. In spite of this difficulty, it was possible on the basis of a large series of cross-neutralization tests to distinguish 4 clearly differentiated antigenic types and a possible 5th type among the 8 strains of *R. orientalis* included in the study. The strains and the grouping observed were as follows: 1) *Karp* (from New Guinea); 2) *Queensland* (from Australia), *Raub* (from Malaya), *Seerangayee* (from Malaya); 3) *Calcutta* (from India), *Imphal* (from India); 4) *Gilliam* (from Assam-Burma border); 5) *Pescadores* (from Pescadores Islands). These results augment evidence previously reported by others as to the antigenic heterogeneity of strains of *R. orientalis*, a fact of importance epidemiologically

and from the standpoint of developing vaccines of proper antigenic composition.

Streptococcal anti-hyaluronidase in rheumatic fever. SUSANNA HARRIS (by invitation) and T. N. HARRIS. *Children's Hospital of Philadelphia, Dept. of Pediatrics, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* The occurrence of antibodies to streptococcal hyaluronidase, as measured by the mucin-clot-prevention test, was studied in groups of convalescents from streptococcal infection, in patients with acute rheumatic fever and in the normal population. In preliminary studies the optimal relations of hyaluronidase, the substrate (hyaluronic acid) and neutralizing antibody were determined; the potency of the enzyme as measured by mucin-clot-prevention (*in vitro*) was compared with spreading activity (*in vivo*) in the rabbit's skin; and the serologic specificity of neutralizing antibodies was established by comparative studies against hyaluronidase of testicular, clostridial and pneumococcal origin. It was found that antibodies to streptococcal hyaluronidase occurred with increasing frequency in the normal population from infancy up to the adult age group. Following streptococcal infection there was an increase in the titer of antibodies to streptococcal hyaluronidase and in acute rheumatic fever the titer was found to be still higher. In a comparative study of the antibody response to 4 streptococcal antigens, hyaluronidase, hemolysin, and 2 somatic fractions, the cytoplasmic particles and supernate proteins, it was found that the mean titers to all of these antigens were elevated in both rheumatic and streptococcal infection. The mean titer was somewhat higher in rheumatic than in streptococcal infection, in the case of 3 of these antibodies. In the case of the 4th, anti-hyaluronidase, this difference was considerably greater. The anti-hyaluronidase titer showed better correlation with changes in the activity of the rheumatic infection than did the other tests. There was, however, no striking correlation between this titer and the severity of the illness. The possible implications of these data, both diagnostic and etiologic, are discussed.

Histochemical evidence for the synthesis of protein by lymphocytes following parenteral injection of antigen. T. N. HARRIS and SUSANNA HARRIS (by invitation). *Children's Hospital of Philadelphia, Dept. of Pediatrics, School of Medicine, Univ. of Pennsylvania, Philadelphia, Penna.* Recent developments in histochemistry have indicated that cells which are actively forming new protein are characterized by large amounts of ribonucleic acid in their cytoplasm. Since there is considerable immunologic evidence of the production of antibody by the popliteal lymph node of the rabbit following the injection of antigens in the foot pad, and since a tissue in which antibody is being formed could be expected to show evidences of protein synthesis, it seemed feasible to attempt to identify the cells involved by histochemical methods. A study was therefore undertaken of the localization and concentration of ribonucleic acid in the cells of the popliteal lymph node in correlation with the production of antibody by that node. Antigens were injected into the foot pads of rabbits, and at various intervals thereafter the draining lymph nodes were examined histologically and chemically. Diffuse hyperplasia was found in the cortex of the lymph node, involving transitional forms between reticulum cells and lymphocytes, as well as young (large) lymphocytes. Large numbers of these cells showed pyronine staining granules (ribonucleic acid) in the cytoplasm, which were abolished by previous incubation of the sections with protease-free ribonuclease or with hot trichloracetic acid solution. Pyronine-staining nucleoli and crescents of cytoplasm were also seen in these cells. Chemical analysis of the nodes showed an increase in ribonucleoprotein but not in desoxyribonucleoprotein. These changes reached a maximum intensity just before the time of maximal antibody content of the node.

Dissociation of antigen-antibody complexes and their bond energy. FELIX HAUROWITZ and LALE ETILI (by invitation). *Dept. of Chemistry, Indiana Univ., Bloomington, Indiana, and Dept. of Medical Chemistry, Istanbul, Turkey.* Experiments: 4.5 ml. rabbit immune serum against anthranil-azo-beef serum globulin, mixed with 10 mg. antigen, kept at 37° for 30 minutes and at −12°C. for 24 hours, gave 27.8 mg. precipitate, containing 3.0 mg. antigen. No precipitate resulted with 150 mg. antigen. When the precipitate produced by 10 mg. antigen was kept at 37° for 30 minutes with 140 mg. antigen, a considerable part was dissolved. The weight of the undissolved precipitates (in bracket antigen) was 9.5 (3.7), 8.8 (3.3) and 12.1 (4.6) mg. in 3 experiments. The precipitates were isolated and analyzed as described previously (J. Immunol. 43: 331, 1942).—Results: 6 of the 8 antibody molecules bound per antigen molecule in the original precipitate are split off by the antigen excess. No antigen is split off.—It is assumed that the irreversibly bound antibody molecules are attached to antigen molecules by the whole area of the determinant site. If an area of 50 $Å^2$ is assumed for the bis-azophenylcarboxylic group and 100 $Å^2$ for the species-specific group of the globulin, a total bonding area of 150 $Å^2$ results, corresponding to 14 water molecules per layer. The bond energy between antigen and antibody must be higher than that of the water displaced from this area. If it is assumed that only 1 layer of water molecules is displaced, a bond energy of $\sim$ 70 kcal. 1 antigen-antibody bond or $\sim$ 0.47 kcal. 1 $Å^2$ results. The loose linkage of those antibody molecules

which are split off by the antigen excess is attributed to steric hindrance by the firmly bound antibody molecules and/or to imperfect complementariness of the specific sites. Both factors cause increase of the distance between bonding groups. If this distance increases from 3 to 4, 5 or 6 Å, the bond energy will decrease from ~ 0.47 to approximately 0.25, 0.15 and 0.10 kcal./Å^2 respectively.

Indirect complement-fixation test for psittacosis-lymphogranuloma venereum group antibodies in chicken antisera. M. R. HILLEMAN and R. J. HELMOLD (introduced by ANNABEL WALTER). *Dept. of Virus and Rickettsial Diseases, Army Medical Dept. Research and Graduate School, Army Medical Center, Washington, D. C.* It is well established that antisera prepared in certain mammals and birds are incapable of fixing complement in the presence of homologous antigen even though antibody can be demonstrated in them by other techniques. Such sera may contain blocking antibodies which, in the presence of homologous antigen, prevent fixation of complement by ordinary antibodies that are capable of binding complement. Sera from chickens immunized against viruses of the psittacosis-lymphogranuloma venereum group do not fix complement in the usual type of test. Such sera have now been shown to possess blocking antibodies which interfere with the binding of complement by mammalian antibodies in the presence of homologous antigen. The technique of the indirect complement-fixation test has been used to determine whether under these conditions chicken antisera provide the broad group reaction displayed by mammalian antisera in complement-fixation tests with this group of agents, or whether they follow the highly strain specific reaction found when chicken antisera are used in neutralization tests. It would appear that when the indirect complement-fixation technique is employed, the chicken sera approach the broad type of group reaction characteristic of mammalian sera in direct fixation tests with this group of viruses.

Tissue reaction to foreign particulate matter altered by presence of adsorbed antigen. HOWARD C. HOPPS and ALFRED M. SHIDELER (by invitation). *Dept. of Pathology, School of Medicine, Univ. of Oklahoma, Oklahoma City, Okla.* Rabbits were given intradermal injections of finely particulate aluminum hydroxide, activated carbon, magnesium silicate (talc) and silicon dioxide (flint). Tissue reactions were studied at 7, 14, 28 and 56 days. A second series of intradermal injections was made, similar to the first, with the exception that horse serum was absorbed upon the particulate matter prior to its administration. Tissue reactions in this group of lesions were compared with those of the former group with special attention to the character of inflammatory exudate, ratio between macrophages and fibroblasts, occurrence of focal necrosis and cellular degenerative changes.

Effect of previous experience with homologous and heterologous types of poliomyelitis virus upon the alimentary carriage of virus by the chimpanzee. HOWARD A. HOWE (by invitation), DAVID BODIAN (by invitation) and ISABEL M. MORGAN. *Poliomyelitis Research Center, Dept. of Epidemiology, The Johns Hopkins Univ., Baltimore, Md.* Eighty oral inoculations have been carried out in chimpanzees with 4 strains of poliomyelitis virus representing 2 major immunologic types, the Brunhilde, Kotter and Frederick strains comprising 1 type, and the Lansing strain the other. The first experience with virus was provided by oral inoculation in the majority of the animals but toward the end of the experiment 14 animals were vaccinated with active Lansing virus given intramuscularly. Evidence of response to the immunization was obtained by testing for virus in stools or for rises in serum antibody titer. The control group consisted of 27 animals of which all responded positively, 2 with paralysis. Challenge was carried out at 1 month or later following first virus contact by the oral administration of titrated pools of rhesus monkey cords. In many instances 2 or 3 homotypic viruses were given to the same animal. Animals which became paralyzed or carried virus in the stools after the first post inoculation week were considered to have shown no evidence of immunity. No animal was considered negative unless no evidence of activity was found in at least 4 stool specimens collected during the 2–4th weeks and tested in 2 monkeys each by intranasal inoculation. The results were as follows: Of 9 animals receiving homologous challenge, 1 subsequently produced virus in the stools. This result differs in no essential respect from the results of 22 homotypic challenges in which 1 animal had virus in the stools on the second week. Twenty-two animals were immunized with Lansing virus either by mouth (8) or by intramuscular vaccination (14). Subsequent oral challenge was carried out with the heterotypic Brunhilde strain, following which 3 animals became paralyzed while 13 showed virus in the stools. Although the tests are not quite complete the infection rate appears to be 15/22 or 68%

Universal serologic reaction with lipid antigen in tuberculosis. REUBEN L. KAHN. *Serology Laby., Univ. Hospital, Ann Arbor, Mich.* It was previously shown that it is possible to demonstrate positive reactions with the serums of normal human beings by special precipitation techniques with tissue extract antigens (Kahn, Kolmer, cardiolipin). A standardized precipitation technique based on different NaCl concentrations, serum dilutions and incubations was then developed with Kahn antigen, known as universal serologic technique. With this technique normal human beings

(giving negative Kahn reactions for syphilis) give precipitation reactions of characteristic serologic patterns. It was observed that, in Kahn-negative patients with acute tuberculosis, precipitation in the universal reaction is increased above the normal level and, as the patients gradually improve, precipitation is correspondingly decreased. This finding indicates that the universal serologic technique might prove helpful as an indicator of tuberculous activity. No increase in precipitation above the normal has been noted in far advanced and terminal cases of tuberculosis.

Development of the specific antibody for a distinctive constituent of Brown-Pearce carcinoma cells. BERNARD KALFAYAN (by invitation) and JOHN G. KIDD. *Dept. of Pathology, New York Hospital, Cornell Medical Center, New York City.* Certain rabbits implanted with Brown-Pearce tumor cells develop an antibody that reacts specifically with a distinctive constituent of them, as previous work has shown (J. Exper. Med. 71: 335, 351, 1940). In ordinary market-bought hybrid rabbits the specific Brown-Pearce antibody was frequently masked by the presence of induced tissue antibodies, which react with extracts of a variety of normal and neoplastic rabbit tissues (J. Exper. Med. 82: 21, 1945), though about half of the inbred 'blue-cross' rabbits implanted with Brown-Pearce tumor cells developed the specific antibody in high titer, usually without the concomitant development of the induced tissue antibodies (J. Exper. Med. 82: 41, 1945). In recent experiments the specific Brown-Pearce antibody appeared in the blood of every one of 17 Havana and 23 Chocolate-Dutch (Havana x Dutch hybrids) rabbits implanted with the tumor cells, often reaching titers of 1:512 or higher. The induced tissue antibodies, by contrast, were present in titers of 1:8 or less in a few specimens but were entirely absent from most of them. Normal rabbits of both breeds provided sera that were devoid of the specific Brown-Pearce antibody and of the induced tissue antibodies.

Quantitative measurement of antibody to a whole bacterial antigen. MARIAN KOSHLAND (introduced by WILLIAM BURROWS). *Dept. of Bacteriology and Parasitology, Univ. of Chicago, Chicago, Ill.* A quantitative method for the measurement of the antibody response to a whole bacterial antigen, the cholera vibrio, has been developed. The method depends upon the colorimetric determination of specific complement fixation using the 50% hemolytic unit. The excess complement procedure, proposed by Heidelberger and his co-workers, was employed in this study, and was adapted to the requirements of a whole bacterial antigen. The results obtained using a whole bacterial antigen have been compared from a theoretical and practical point of view with those involving viral or bacterial extract antigens. The method has been used to determine the antibody levels produced in the serum and the feces of guinea pigs, either actively immunized with the cholera vibrio or passively immunized with anti-cholera rabbit serum.

Equilibrium studies on the reaction of antibody with hapten. LEONARD LERMAN (introduced by DAN H. CAMPBELL). *Gates and Crellin Laby. of Chemistry, California Inst. of Technology, Pasadena, Calif.* Quantitative studies have been made on the combination of antibody with a simple monohaptenic substance. Antibody was specifically purified from rabbit antiserum homologous to the p-azophenylarsonic acid hapten by precipitation with a simple trihaptenic antigen, followed by dissociation of the washed precipitate with a monohaptenic substance. A cellophane bag containing the purified antibody solution was immersed in a buffered solution of normal rabbit gamma-globulin (at the same protein concentration) together with a dialysable monohaptenic dye. After continuous agitation at constant temperature for about 24 hours the dye concentrations in the 2 solutions were measured colorimetrically to obtain the equilibrium composition in terms of bound and unbound hapten. From measurements at varying ratios of hapten to antibody the free energy of combination, the maximum binding capacity, and related quantities have been determined. Under various conditions the free energy change is of the order of -7.5 to -8.0 kcal. per M of hapten bound.

Some properties of purified diphtheria toxoid. LEO LEVINE and LOUISE WYMAN (introduced by GEOFFREY EDSAL). *Antitoxin and Vaccine Laby., Massachusetts Dept. of Public Health, Boston, Mass.* Crude toxoid, obtained as the detoxified filtrate of diphtheria bacillus culture grown on Mueller's semi-synthetic medium, modified in this laboratory, has been purified by a simple 2-stage salt fractionation, and has been evaluated with respect to purity, antigenicity, and stability. Preparations have been obtained assaying 2100 Lf/mg. of protein nitrogen. Preliminary laboratory studies have indicated that stability of the toxoid protein is impaired by addition of glycine to the diluent in the usual quantities, whereas gelatine appears to increase stability. Injections of 11 Lf have protected guinea pigs against the standard challenge dose of 10 M.L.D. Potency tests with varying amounts of antigen indicate that the antigenicity per Lf unit increases as the purity is increased. A linear relationship has been observed between the logarithm of the flocculation time (Kf) and that of the dilution (or of the Lf) at the equivalence point. These observations support the evidence of previous workers that a relationship may be established between antigenic efficiency and flocculation time. This product is now on clinical trial; the practical significance of the findings presented will be discussed.

Allergenic fraction of ragweed pollen prepared by electrophoresis-convection (Nielsen-Kirkwood cell). MARY HEWITT LOVELESS and ROBERT BEST (by invitation). *New York Hospital and the Dept. of Medicine, Cornell Univ. Medical College, New York City.* The principle of electrophoresis-convection has been applied to the problem of fractionating ragweed extract, with the eventual aim of obtaining a single allergen from pollen. The Nielsen-Kirkwood apparatus affords a gentle means of separation and larger yields of fraction than are available from the Tiselius cell used by Abramson. Preliminary results with products obtained from one or more runs in the Nielsen-Kirkwood apparatus reveal that the electrophoretically immobile material (called 'top' fraction) is simplified over the original extract. This in evidenced by a) the almost complete removal of color from this fraction, b) reduction in precipitable nitrogen as well as in carbohydrates, c) a decrease in reagin-binding activity roughly related to its loss of precipitable nitrogen, d) immunological crosstests which showed removal of some antigenic constituents. Despite the purification of 'top' fraction, it uniformly elicited the characteristic immediate responses in allergic skin and conjunctiva, as well as producing the typical Prausnitz-Kustner reaction with pollen-allergic sera. Occasional sera were encountered which contained reagins for only those antigens which remained in the 'top' fraction. Patients who were given a course of therapeutic injections with 'top' fraction produced 'protective,' or thermostable, antibodies in their blood streams and showed increased tolerance toward the fraction when tested intracutaneously and by conjunctival test. Furthermore, a significant proportion of them proved to be resistant clinically when exposed to natural pollination. This clinical immunity tended to be selective since some individuals responded much better to the fraction than they had to whole extract in prior years, while for others the reverse was true.

New observations on an autoagglutinin. H. H. LUBINSKI and D. MENDEL (by invitation). *Pathological Laby. and Dept. of Medicine, Jewish General Hospital, Montreal, Canada.* The serum of a patient suffering from hemolytic anaemia and belonging to Blood Group A contained an autoagglutinin which had the following characteristics: When serum dilutions and red cell suspensions were made with normal saline, clumping of red cells was observed at room temperature, but not at 37°C; clumps dissolved if warmed to incubator temperature. When, instead of saline, 30% beef albumin solution was used, agglutination also occurred at incubator temperature. Using human serum or plasma, clumping occurred only at room temperature. The autoantibodies could be adsorbed on human cells of Group A or O, and transferred into normal saline with the technique described by Landsteiner and Miller, even when the whole process was perfected in an incubator room at 37°C. These purified agglutinin solutions reacted with human red cells, suspended in saline, at room temperature, but not at 37°C.; red cells suspended in albumin were clumped at 37°C. as well. The agglutinin solution, furthermore, agglutinated and hemolyzed rabbit red cells suspended in saline at both temperatures.

Relation of complement to thermolabile hyaluronidase inhibitor of serum. STANLEY MARCUS and JOHN K. FULTON (by invitation). *Rackham Arthritis Research Unit and Dept. of Medicine, Univ. of Michigan, Ann Arbor, Mich.* A modification of a biological technique (streptococcal decapsulation) for the quantitative determination of hyaluronidase using testicular or streptococcal enzyme has been used. By this method and turbidimetric assay also, it was found that sera heated at 56°C. for 20 minutes lost their enzyme inhibiting capacity. Activity of the enzyme inhibitor decreased during refrigeration. These findings suggested an analogy to complement (C′) function. Loss of inhibition following heating was replaced by addition of lyophilized guinea pig C′. While fresh guinea pig serum contained inhibitor, reconstituted lyophilized serum did not. It was concluded that inhibition depends upon a thermostable component lost upon lyophilization and a thermolabile component surviving this process. Pooled guinea pig serum was fractionated to yield C′3 and C′4 deficient C′. Both C′3 and C′4 deficient fractions lacked enzyme inhibitory capacity. Although heated guinea pig serum (C′3, C′4) plus ammonia or zymin-treated serum did not show inhibitory capacity, heated serum plus ammonia and zymin-treated fractions of C′ had fully restored inhibitory ability. It has been assumed, therefore, that serum inhibitor of hyaluronidase depends upon the presence of the 4 characterized C′ components plus still another thermostable component. Although it is demonstrable by turbidimetric procedure that heated serum does not inhibit enzyme action, preliminary data indicate that C′ will not reactivate the thermolabile inhibitor measured by this technique. This is believed to be further evidence that different procedures may measure different stages in the hydrolysis of hyaluronic acid. There was no fall in inhibitor concentration of guinea pig serum following anaphylaxis whereas C′ concentration was decreased. C′ concentrations of human sera could not be correlated with inhibitor titers. The implications of these findings will be discussed.

Effects of gliotoxin and other sulfur-containing compounds on tumor cells *in vitro*. JOHN W. MASON (by invitation) and JOHN G. KIDD. *Dept. of Pathology, New York Hospital, Cornell Medical Center, New York City.* Gliotoxin, held in brief contact with tumor cells *in vitro*, inhibits their subsequent

proliferation upon implantation in susceptible hosts, as previous work has shown (Science 105: 511, 1947). Two other compounds having unusual sulfur linkages (4-acetamido-5,5-dimethyl-1,2-dithiolane-3-one and 4 methyl-5-methylimino-1,2-dithiazolidone-3—synthesized and made available by Professor John R. Johnson) have likewise proved highly effective against lymphosarcoma cells in mice, inhibiting their growth after 1 hour's contact *in vitro* in concentrations of 1–5 μg/cc., the effective level of gliotoxin in concurrent tests being 1 μg/cc. or slightly less. Two additional compounds of similar sort (4-benzamido-5,5-dimethyl-1,2-dithiolane-3-one and 4-methyl-5-methylimino-3-thio-1,2-dithiazolidone-3), were somewhat less soluble, but were also inhibitory in low concentrations. Desthiogliotoxin had no effect on tumor cells in concentrations of 800 μg/cc. Simple monothiol compounds (cysteine, glutathione, and monothioglycerol) had no inhibitory effect on the lymphosarcoma cells in concentrations up to 800 μg/cc., while two dithiols, BAL and dithiobiuret, were inhibitory at 100–200 μg/cc. When mixtures containing low concentrations of gliotoxin and high concentrations of the mono- and dithiol compounds were allowed to interact for a few minutes and then tested against tumor cells, the inhibitory effect of the gliotoxin was diminished or abolished. This effect of glitoxin was not modified, however, when very large amounts of cysteine, glutathione, monothioglycerol and BAL were brought into contact with tumor cells that had previously been exposed for 1 hour to gliotoxin in a concentration of 10 μg/ml.

Immunogenic difference between two related strains of poliomyelitis virus. Joseph L. Melnick. *Section of Preventive Medicine, Yale Univ. School of Medicine, New Haven, Conn.* By means of vaccination and neutralization tests the 2 strains of poliomyelitis virus—Lansing and Y-SK—are immunologically related. The Lansing strain, isolated in the monkey from the CNS of a fatal case in Lansing, Mich., in 1937, was successfully established by Armstrong in cotton rats and mice. When used in the present experiments, the strain had been through 260 serial passages in mice and still retained its pathogenicity for monkeys. The Y-SK strain, isolated by Trask and Paul from the stools of an abortive case in New Haven, Conn., in 1937, was subsequently found to produce in mice, cotton rats, and hamsters a myelitic disease. The virus transferable in these rodents was shown to be immunologically the same as the parent simian strain. At the time it was used for these experiments, this strain had been passed through 20 transfers in monkeys and 15 in cotton rats and mice. It too remained pathogenic for primates. Previous work had established that single or multiple feedings of the Y-SK virus to cynomolgus monkeys usually result in the production of antibodies either by the first day of paralysis or by the 4th week in the asymptomatic animals. When the Lansing virus was fed repeatedly to a group of cynomolgus monkeys, none of them responded either by apparent disease or by the development of neutralizing antibodies. When these same monkeys were then fed Y-SK virus, they again remained in apparent good health, but they were now found to have developed neutralizing antibodies. Subsequent challenge by the intranasal route indicated an appreciable degree of resistance among the group. Thus in contrast to the Lansing strain of poliomyelitis virus, the homotypic Y-SK strain is capable of engendering antibodies when it is administered orally to cynomolgus monkeys and such monkeys may have an increased resistance to virus instilled intranasally.

Resistance to endotoxins of enteric bacilli in patients convalescent from typhoid and paratyphoid fever. Herbert R. Morgan and Franklin A. Neva (introduced by Thomas Francis, Jr.). *Dept. of Epidemiology, School of Public Health, Univ. of Michigan, Ann Arbor, Mich.* Patients convalescent from enteric fevers due to infection with *Salmonella typhosa* or *S. paratyphi* were shown to be more resistant than normal individuals to the pyrogenic and general systemic effects of the toxic, somatic antigens (endotoxins) of *S. typhosa* or *Shigella dysenteriae* following their intravenous administration. The significance of these findings in regard to the clinical course of the disease and the process of recovery will be discussed.

Precipitin tests for the diagnosis of Chagas' disease, with special reference to the specificity of polysaccharides. A. Packchanian and L. Turck (by invitation). *Dept. of Bacteriology and Laby. of Microbiology, Univ. of Texas, School of Medicine, Galveston, Texas.* *Trypanosoma cruzi* antigens for diagnosing Chagas' disease by the precipitin test were prepared from washed, packed *Tr. cruzi* grown *in vitro*. The cytolysis of the trypanosomes was accomplished by various means, and the active principle for the precipitin test was extracted by several methods. The first antigen, a crude aqueous extract, was found to be specific and gave positive precipitin reactions with both *Tr. cruzi* antiserum and sera from known human and animal cases of Chagas' disease. Inasmuch as the precipitin titer of this aqueous antigen was low, further studies were conducted to isolate from the cellular substance of *Tr. cruzi* the active component, which proved to be the polysaccharide complex. Several well known immunochemical methods of isolating polysaccharides from microorganisms, such as Avery's, Felton's, Fuller's and Sevag's methods, were employed in the preparation of *Tr. cruzi* 'polysaccharide antigens'. The specificity and potency of these antigens were determined and compared. These antigens gave much higher precipitin titers than the crude aqueous extract. Quantitative studies of serological

cross-reactions between the *Tr. cruzi* antigens and several antisera and serum samples from other trypanosomiases, leishmaniases and bacterial infections were made. Likewise, polysaccharides isolated from several species of trypanosomes, leishmania and bacteria were tested with *Tr. cruzi* and homologous antisera. The relative merits of the *Tr. cruzi* antigens for the precipitin test in the diagnosis of Chagas' disease have been evaluated.

Amino acids, analogues and the propagation of Theiler's GD VII virus in mouse brain tissue culture. HAROLD E. PEARSON and RICHARD J. WINZLER (by invitation). *Depts. of Bacteriology and Biochemistry, Univ. South. Calif. School of Medicine and the Laby. Div., Los Angeles County Hospital, Los Angeles, Calif.* The effects of various amino acids and certain analogues on the propagation of the GD VII strain of Theiler's mouse encephalomyelitis virus in cultures of minced, one day mouse brain were studied. Tissue suspended in Simm's solution at *p*H 8–9 and contained in 50 ml. Erlenmeyer flasks was incubated for 2–3 days at 35°C. The titer of virus attained in cultures as tested by intracerebral injection of mice was reduced slightly by the presences of 1 mg/ml. L-lysine monohydrochloride but not by L-arginine monohydrochloride, L-cystine, L-cysteine hydrochloride, glycine, L-histidine monohydrochloride, L-leucine, L-proline, DL-alanine or DL-phenylalanine. The titer was markedly reduced by the presence of 3–5 mg/ml. DL-ethionine, beta-2-furyl-DL-alanine or beta-2-thienyl-DL-alanine; the addition of DL-phenylalanine in the same concentration did not reverse the actions of the last 2 compounds. Glutathione 5 mg/ml. but not 1 mg/ml. also reduced the titer of virus in cultures.

Effect of lithium periodate on crystalline bovine serum albumin. GERTRUDE E. PERLMANN (by invitation) and WALTHER F. GOEBEL. *Labys. of The Rockefeller Inst. for Medical Research, New York City.* When certain biologically active proteins are subjected to the action of dilute lithium periodate at physiological values of *p*H, their activities are rapidly altered and finally destroyed. In order to gain an insight into the nature of the chemical changes which occur, a study has been made of the action of 0.03 M lithium periodate on crystalline bovine plasma albumin. It has been found that after contact with the reagent the ultraviolet absorption spectrum as well as the electrophoretic behavior of the protein has been radically altered. In addition, the solubility of the protein in salts has been changed, as has its solubility in the acid range of *p*H values. At a *p*H of approximately 3.5 the protein is entirely insoluble, but dissolves as the solution becomes more acid. The cystine, cysteine and tyrosine content of the protein has been greatly lowered, and the tryptophane appears to be completely destroyed following treatment with 0.03 M lithium periodate at room temperature over a period of 72 hours. These chemical and physicochemical changes are accompanied by a complete loss in the ability of the oxidized protein to incite antibodies in experimental animals, yet the changes are not so profound as to cause loss in precipitability of the oxidized albumin in an antiserum prepared against the native protein.

'Nonspecific' complement-fixing activity of cloudy avian sera. CHRISTINE E. RICE. *Div. of Animal Pathology, Science Service, Dominion Dept. of Agriculture, Animal Diseases Research Inst., Hull, Quebec, Canada.* Whereas inactivated chicken and turkey sera do not ordinarily fix complement with homologous bacterial or viral antigens, an atypical type of complement-fixing activity has been observed with higher dilutions of sera collected from birds during periods of active egg-production. Since fixation of complement was recorded with normal as well as immune sera and was noted with various unrelated antigens, it appeared to be 'nonspecific' in nature. The fact that fixation was most pronounced with cloudy sera suggested that serum lipids may have played some rôle in the reaction. The results of chemical analyses seemed to support this supposition. The possible relationship of such activities to certain 'nonspecific' reactions encountered in the serologic diagnosis of human infection will be discussed.

Relationship between catalase activity and virulence in pasteurella pestis. MORRIS ROCKENMACHER (introduced by A. P. KRUEGER). *Office of Naval Research Task V, Dept. of Bacteriology and U. S. Naval Medical Research Unit No. 1, Univ. of California, Berkeley, Calif.* The catalase activity of virulent and avirulent strains of *Pasteurella pestis* was measured by an adaptation of the procedure of Huddleson (Univ. of Michigan Technical Bulletin 182: January 1942). Optimal conditions for enzyme activity in these organisms, as shown by a study of the kinetics of the reaction, were used. Catalase activity was expressed as ml. of 0.1 N hydrogen peroxide decomposed in 60 minutes by a standardized suspension of organisms. A minimum of 2 separate measurements was made for each strain. Enzyme activity appeared to follow the curve for a mono-molecular reaction. The catalase activity of virulent strains was greater than that of avirulent strains. In the 14 virulent strains studied, enzyme activity ranged from 12.9 to 19.9 ml., with a mean value of 16.7 ± 0.4. For 11 avirulent strains, values of 2.1 to 8.6 ml. were found, with a mean value of 5.1 ± 0.5. The difference between the mean values of the virulent and avirulent organisms was significant. Virulence titrations were simultaneously performed by the subcutaneous inoculation of the suspension of organisms used for catalase testing into 7- to 12-week old mice. The resultant LD50 values ranged from 6 to

5000 virulent organisms. Avirulent strains caused no deaths when 20,000 organisms were injected subcutaneously.

Intradermal antibody-antigen and antigen-antibody reactions in persons having poliomyelitis, contacts and non-contacts in relation to poliomyelitis. EDWARD C. ROSENOW. *Bacteriological Research, Longview Hospital, Cincinnati, Ohio.* The solutions of thermal antibody and of antigen injected intradermally consisted of the bacteria-free supernatants of saline suspensions of streptococci isolated in studies of poliomyelitis. The former after autoclaving suspensions containing twenty billion streptococci/ml. for 96 hours and adding an equal volume of saline containing 0.4% phenol; the latter after heating suspensions containing ten billion streptococci/ml. at 70°C. for one hour and adding 0.2% phenol. Three hundredths ml. of each and of control solutions were injected into the skin of the anterior aspect of the forearm of 432 persons having poliomyelitis, of 434 contacts and 2193 non-contacts. Reactions of 5 sq. cm. or more to 'poliomyelitis' streptococcal thermal antibody indicating specific antigen and a corresponding streptococcal infection occurred in all persons having poliomyelitis, in nearly all contacts and non-contacts during September and in almost no one in spring and winter remote from poliomyelitis. Reactions indicating antigen were greatest, alike in males and females, and reactions indicating antibody were least at the onset of poliomyelitis. Antigen diminished and antibody increased during recovery far more rapidly in persons having mild attacks than in persons severely paralyzed and antibody titers were uniformly higher in females than in males corresponding to the lower incidence of and mortality from poliomyelitis in females. 'Poliomyelitic' streptococcal antibody titers in September were strikingly greater in persons recovering, in persons that had recovered from mild poliomyelitis and in well persons that had previously been exposed to epidemic poliomyelitis than in corresponding persons not previously exposed.

Antigenic relationship of dengue and yellow fever viruses with those of west-Nile and Japanese B encephalitis. ALBERT B. SABIN. *The Children's Hospital Research Foundation, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio. Rhesus* monkeys inoculated intracerebrally with human serum containing various immunological types of dengue virus (Hawaii, New Guinea 'B,' 'C' or 'D') developed complement-fixing antibodies not only for the Hawaii dengue virus (benzene-extracted 20% mouse brain antigen) in titers of 1:64 to 1:128 but also for the Japanese B and West Nile viruses in titers of 1:16 to 1:64 and to a lesser degree for the virus of yellow fever, but not for the St. Louis, Western equine or Rift Valley fever viruses. The serum of human volunteers residing in the U. S. A. who recovered from infection with the Hawaii type of dengue virus by mosquito bites or inoculation of human serum, also had C-F antibodies for the Japanese B, West Nile and yellow fever viruses, but not for the St. Louis, Western equine, or Rift Valley fever viruses. Rhesus monkeys inoculated with yellow fever virus (French neurotropic or Asibi viscerotropic strains or both) which developed C-F antibodies for the yellow fever virus in titers of 1:64 to 1:256 also regularly developed C-F antibodies for the Japanese B and West Nile viruses in titers of 1:4 to 1:32 and occasionally also for the Hawaii dengue virus, but not for the St. Louis, Western equine or Rift Valley fever viruses. A Japanese B antiserum prepared in mice with a homologous C-F titer of 1:256, had a titer of 1:128 with West Nile, 1:32 with yellow fever, 1:4 with Hawaii dengue, 1:2 with St. Louis encephalitis virus, and yielded negative results with similar antigens prepared from the Western equine and Rift Valley fever viruses. The common antigen (or antigenic groups) possessed by the dengue, yellow fever, Japanese B encephalitis and West Nile viruses is present in low concentration and can be demonstrated only in the most potent preparations, although it is not necessarily proportional to the number of homologous antigenic units. Thus, while a Japanese B antigen with 128 homologous units had only 4 units when tested with a dengue antiserum, the West Nile antigen with 16 homologous units was as active as the Japanese B antigen in its reaction with the antisera for the related viruses.

Differences in antigenic capacity among influenza virus strains. JONAS E. SALK, ANGELA M. LAURENT (by invitation) and RACHEL C. MCGINNIS (by invitation). *Virus Research Laby., Dept. of Bacteriology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Penna.* In the course of studies carried out in 1947–48 employing influenza virus vaccines containing the FM1 strain (Type A-prime), it was observed that the antibody response in man to the FM1 component of the vaccine was noticeably less than was the response to the other two components, PR8 (Type A) and Lee (Type B). For this reason studies were undertaken to investigate the hypothesis that strains of influenza virus differ in their antigenic capacity. Because of the importance of this question, in relation to continuing field studies of vaccination, a study was made, in man as well as in experimental animals, of the antigenic capacity of selected strains. The studies in man were carried out by inoculating groups of individuals with univalent vaccines containing comparable amounts of virus hemagglutinin. Determinations were then made of the titer, in the pre- and two-week post-vaccination sera, of hemagglutination-inhibition antibody, measured with homologous and heterologous antigens. Similarly, groups of mice were inoculated intraperitoneally

with the univalent vaccines, diluted serially, to determine antigenic extinction titer. The latter is an index of the highest dilution of vaccine that is capable of inducing, in the mouse, the formation of neutralizing antibody measured *in ovo*. Both in man and in the mouse, marked differences were found in the effectiveness of different strains in calling forth antibody. It was of further interest that different lines of the same strain differed in this respect. The implication is evident of the significance of these findings in relation to the problem of selecting strains of virus to be included in vaccines for use in man.

Loss of hemolytic complement and of granulocytes following reinjection of an antigen into the rabbit. A. B. STAVITSKY (by invitation), R. STAVITSKY (by invitation) and E. E. ECKER. *Inst. of Pathology, Western Reserve Univ., Cleveland, Ohio.* The decrease of complement observed in anaphylaxis, serum sickness and certain infectious diseases has been attributed to its fixation by an antigen-antibody complex *in vivo*. To determine whether such fixation occurs, the following experiments were performed. Various antigens were injected into rabbits and their serum complement estimated immediately before and after the intravenous reinjection of each antigen. Thirty and 60 minutes after the reinjection of the antigens, such as ovalbumin, etc., a great reduction of hemolytic complement activity was noted. This reduction occurred both in the presence and absence of anaphylaxis. Since it did not occur in the absence of extracellular antibody it is most likely that complement was bound by an extra-cellular antigen-antibody complex *in vivo*. A similar mechanism may be assumed to mediate the loss of complement in allergic and infectious diseases. The 'Complement Reduction Reaction' may prove to be useful for the detection of extracellular antigens and antibodies. It is highly specific and sensitive, apparently unaffected by excess antigen or antibody, and may require relatively little antigen for its production; but is not as yet quantitative. A granulocytopenia generally accompanies the drop in complement. This response appears to be related to anaphylactic and anaphylactoid reactions as well as to an *in vivo* antigen-antibody combination. This response may be employed for the early detection of the anaphylactic type of hypersensitivity to a given antigen. The significance of the loss of antibody, complement and granulocytes during the early part of the 'negative phase' in immunization deserves further clarification.

Antigen-antibody reaction of intact leukocytes: 1. Plasma cell; 2. Variable specificity of maturing cells. BERNHARD STEINBERG and RUTH A. MARTIN (by invitation). *Toledo Hospital Inst. of Medical Research, Toledo, Ohio.* We indicated previously (Proc. Soc. Exp. Biol. & Med. 56: 50–52, 1944; *J. Immun.* 51: 421–426, 1945; 52: 71–75, 1946; 53: 137–141, 1946) that various types of mature and immature INTACT leukocytes possess specific antigenic substances and that each cell type can be demonstrated by agglutination. Normal and leukemic leukocytes were found to be antigenically similar, whereas cells from lymphosarcoma were antigenically dissimilar from those of lymphatic leukemia. These findings suggest an immunochemical difference between normal and leukemic cells on one hand and malignant cells on the other. In the present study, the antigenic similarity of the plasma cell in relation to other leukocytes was investigated. In addition, leukocytes of various stages of immaturity were tested for antigenic specificity. Antisera were developed for granulocytes, lymphocytes, monocytes and plasmocytes of mature and of several phases of immaturity. Agglutinations of intact leukocytes indicated individual variations but with a group specificity for all immature forms of a given type of cell. Plasmocytes were found to be antigenically dissimilar from granulocytes, lymphocytes and monocytes.

Metabolism of Brown-Pearce rabbit carcinoma cells in normal and immune serum. W. H. SUMMERSON (by invitation) and JOHN G. KIDD. *Depts. of Biochemistry and Pathology, Cornell Univ. Medical College, New York City.* The metabolism of viable Brown-Pearce rabbit carcinoma cells was studied in the presence of normal rabbit serum and of rabbit serum that contained high titers of the antibody that reacts with a distinctive constituent of the Brown-Pearce cell. For the immune sera, the relation between the number of cells and the volume and titer of serum used was such that an excess of the specific antibody was present. Measurements were made of oxygen consumption, R.Q., aerobic and anaerobic glycolysis, glucose utilization, and lactic acid production, over a 3 to 4-hour period, under physiological conditions of temperature and *pH*. No significant effect of the immune sera on these metabolic characteristics of the Brown-Pearce cell was evident, even though control bioassays demonstrated that incubation of the cells with immune sera under conditions similar to those used in the metabolism studies rendered the cells incapable of giving rise to new tumors when implanted in susceptible host rabbits.

Antigenicity of staphylocoagulase. MORRIS TAGER and HARRIET B. HALES (by invitation). *Dept. of Bacteriology, Yale Univ. School of Medicine, New Haven, Conn.* Although the antigenicity of coagulase has been a matter of controversy, interest in this problem has been reopened by studies indicating that many human sera inhibit coagulase activity, that similarly most plasmas show varying degrees of resistance, and that changes in inhibitory properties of sera may follow staphylococcal infection. Using standardized and

concentrated coagulase purified 300-fold (activity to nitrogen ratios) and showing 2 mobile components on electrophoresis, rabbits were inoculated with *a*) coagulase alone; *b*) coagulase plus Wood 46 alpha lysin as adjuvant; and *c*) the lysin only. Five of 6 test rabbits developed an increase in serum neutralization, increased plasma resistance to coagulase action, complement fixing bodies, and agglutination of coagulase-coated collodion particles. The 3 controls and 1 test animal failed to respond. The antigenicity of the coagulase, and the specificity of the responses observed, is further supported by such considerations as the high degree of purity of the injected coagulase, the consistently better response of the animals treated with the adjuvant toxin as well as the coagulase, by the sharp anamnestic reaction following a single booster dose, and by the specific removal of neutralizing bodies by adsorbtion on coagulase-coated collodion particles. Although the findings warrant the conclusion that antibodies were produced to coagulase in 5 or 6 rabbits, there are data indicating that coagulase activity may be blocked by other mechanisms as well.

X-irradiation on antibody decline. WILLIAM H. TALIAFERRO and LUCY G. TALIAFERRO (by invitation). *Dept. of Bacteriology and Parasitology, Univ. of Chicago, Chicago, Ill.* Changes in hemolysin titer have been followed by photometric methods in 3 series of rabbits after receiving per kg.: *1*) 2 x 10^7 sheep red cells in one injection; *2*) 2 x 10^8 cells in one injection; and *3*) 17 x 10^9 cells in 12 injections. After the antibodies began to decline, half of each series was irradiated twice (1 or 2 weeks apart) with 400 r hard X-rays (0.5 mm. copper and 1 mm. aluminum filters). In no animals was there a significant rise in hemolysin titer after irradiation. At this level of irradiation there is marked destruction of lymphocytes but the macrophages are highly phagocytic and show no evidence of injury. In view of the accumulated evidence indicating that lymphocytes contain large amounts of antibody, these negative results raise questions as to *1*) the relative importance of the macrophages and lymphocytes in producing and storing antibodies at this stage of immunization; and *2*) the possibility that the particular method used to destroy lymphocytes may lead to the destruction of antibody.

Tuberculostatic activity of the blood and urine of animals given gliotoxin. RALPH TOMPSETT (by invitation), WALSH MCDERMOTT (by invitation), and JOHN G. KIDD. *Depts. of Medicine and Pathology, The New York Hospital, Cornell Medical Center, New York City.* Two samples of purified gliotoxin, generously provided by Professor Johnson, have regularly inhibited the growth of virulent tubercle bacilli of human origin in concentrations of 0.01–0.04 μg/ml. in various liquid media. These samples were likewise effective against mouse lymphosarcoma cells *in vitro* in concentrations of 1.0 μg/ml. or slightly less, as indicated in the preceding abstract. The purified gliotoxin was much less active against a number of other bacterial species, the inhibitory concentrations ranging from 250 μg/ml. for Ps. aeruginosa to 8.0 and 4.0 μg/ml. for *Str. viridans* and *Pneumococcus* respectively. Concentrations within this range were inhibitory to strains of *Enterococcus*, *Streptococcus* (Group A), *Staphylococcus*, *B. subtilis*, *A. Aerogenes*, and *E. coli*. From five to fifteen minutes after the oral or parenteral administration of gliotoxin to mice, guinea pigs and rabbits, the plasma of the animals inhibited growth of tubercle bacilli in dilutions as high as 1:320, and significant degrees of activity persisted for at least two hours thereafter in most instances. Urine procured from animals of all three species two to three hours after they have been given gliotoxin proved inhibitory to the growth of tubercle bacilli in dilutions from 1:320 to 1:1280. Despite prolonged administration of gliotoxin in doses calculated to provide inhibitory concentrations in the blood for a large part of each day, no favorable influence on the course of tuberculosis in mice was demonstrable.

Inactivation of influenza virus by oxidizing agents. ROBERT R. WAGNER and IRVEN B. STACY, JR. (introduced by LAVERNE A. BARNES). *Naval Medical Research Inst., Bethesda, Md.* The PR8 strain of influenza A virus is inactivated *in vitro* by dilution in various oxidizing agents. Infectivity was determined by inoculation of chick embryos and the hemagglutinating capacity of harvested allantoic fluid. P-benzoquinone, potassium permanganate and sodium periodate were effective in inactivating up to 50,000 I. D. of virus. Oxygen, ammonium iodoxybenzoate and cytochrome C were ineffective and it appears that this inactivation is associated with dehydrogenation. Glucose counteracts the effect of periodate but not of quinone, permanganate or hydrogen peroxide. L-ascorbic acid, in equimolar concentration, is antagonistic to the action of all 4 compounds; 1-cysteine protects only against quinone and peroxide. The degree of activity of these agents is roughly associated with the redox potentials, except for quinone which appears to exhibit greater specificity. These compounds, at 0.001 M concentration, destroy the capacity of the virus to be adsorbed onto chicken erythrocytes; this also correlates with loss of infectivity. However, virus treated with quinone at lower concentrations, although rendered non-infective, will retain its ability to be adsorbed. Furthermore, inactivation by low concentrations of quinone can be reversed by cysteine and glutathione but not by ascorbic acid. These preliminary results indicate that at 0.001 M concentrations these oxidizing agents act as denaturants of PR8

virus; but the site of action of quinone in lower concentrations may be on a sulfhydryl moiety of the virus and that this reaction involves no loss of adsorbability and is reversible.

Antibody against encephalomyocarditis virus in the sera of American wild rats. JOEL WARREN, SUDIE B. RUSS and HELEN JEFFRIES (introduced by JOSEPH E. SMADEL). *Dept. of Virus and Rickettsial Diseases, Army Medical Dept. Research and Graduate School Army Medical Center, Washington, D. C.* In the search for natural reservoirs of the viruses of the encephalomyocarditis group (Columbia-SK, M.M., E.M.C. and Mengo viruses) sera from various species of wild-caught rodents have been examined for the presence of neutralizing antibody for E.M.C. virus. Seventy-nine of 440 sera from *Rattus norvegicus* and *Rattus alexandrinus* possessed considerable amounts of neutralizing substances for E.M.C. virus. Tests performed intraperitoneally in mice showed that such sera were capable of neutralizing 1,000 to 100,000 MLD's of the agent. The geographic distribution of immune rats varied considerably. For example, of 40 rat sera collected in rural areas of the state of Mississippi 39 were positive, whereas only 9 of 75 from Florida and 9 of 64 from Georgia contained this antibody. In the state of California 11 of 32 rats from Orange county were positive while only 2 of 49 from San Diego county had antibody. In general, the incidence of rats with E.M.C. antibody was greatest in southern areas and least in northern regions. Indeed, none of the 38 sera from rats caught in the states of Michigan or Washington or in British Columbia possessed immune substances. None of the sera from a group of 61 rodents made up of jack rabbits, squirrels, marmots and chipmunks contained neutralizing antibody, even though they were trapped in regions where there were a number of E.M.C. immune rats. It appears that wild rats constitute one of the natural reservoirs of the E.M.C. group of viruses.

Role of cathepsin II in the mechanism of caseation in experimental tuberculosis of rabbits. CHARLES WEISS and JULIUS SCHULTZ (by invitation). *Labys. of the Jewish Hospital, Philadelphia, Penna.* One series of rabbits was infected intratracheally with 0.001 mg. of a virulent (Ravenel) culture of *M. tuberculosis* and killed at intervals of from 5 to 20 weeks. A second series was sensitized with 2 successive weekly intravenous injections of 1 mg. of a non-virulent R_1 strain, reinfected intratracheally 2 weeks later with a Ravenel culture, and treated similarly. Sections of lung and kidney showed histologically varying amounts of caseation, usually surrounded by granulomatous tissue. Weighed organs, preserved in a dry ice refrigerator, were homogenized in a Waring blendor and cathepsin II estimated. (Method of estimating hydrolysis of BAA was described by Schultz, J., Jour. Biol. Chem.: 1948, in press). After primary infection with virulent tubercle bacilli, the catheptic activity of certain organs was altered. While there were individual variations, there were statistically significant decreases in the hydrolysis of BAA by the livers and kidneys of rabbits killed 5 weeks after primary infection. The lungs, now weighing 2 to 10 times their normal values, had 2 to 10 times the normal enzymatic activity based on total organ content. The animals which were first sensitized with R_1 cultures and then reinfected with a virulent strain, showed decreases in the liver and marked increases in the kidneys and lungs, if killed 4 to 5 months after reinfection. Thus, the cathepsin II activity expressed as the first order reaction constants or K/gm N, were for normal liver, kidney, and lung: 28 ± 2.1, 24 ± 1.9, and 8.6 ± 0.8 while those for the immunized and reinfected animals were 17 ± 2.1, 38 ± 6.0, and 2.1 ± 2.9, respectively. In explanation the following is suggested: The liver is an organ of high natural immunity to infection with tubercle bacilli, hence, the bacilli of reinfection do not multiply actively there and do not call forth an intense mononuclear infiltration. The kidneys and lungs, on the other hand, possess much less natural immunity, therefore permit a greater multiplication of tubercle bacilli and a much more rapid and intense mobilization of inflammatory cells which are probably the source of much of this added enzyme.

Color reactions with malignant sera. EMIL WEISS. *Tumor Clinic, Michael Reese Hospital, Chicago, Ill.* A number of aniline dyes in dilutions 1:10,000 do not show any color changes on addition of malignant sera. Sera of normal individuals, those afflicted with various diseases or benign tumors turn the original blue color of the same dyes green. The glassware must be clean and free of any acid or dye. The sera must be fresh and thoroughly centrifuged. Hemolyzed or icteric sera can not be used. The dyes are kept in 0.1% stock solutions of rubbing alcohol (70% isopropyl alcohol). The stock solution is diluted with distilled water 1:10 before use. To 0.5 cc. of the diluted dye 0.5 cc. of serum is added, the tube is shaken for a few seconds and the color changes are noted. A positive control containing a known malignant serum and a negative control containing a known normal serum are handled in the same manner. A dye control correspondingly diluted with water also is used. The tubes are placed overnight or for 12 hours in the refrigerator and then the final reading is made. Eventual color changes become more distinct after standing several hours. Over 300 sera were examined with the above technic. Positive (blue) reactions were obtained in 89.1% of malignant sera. Azur II, toluidine blue, trypan blue and victoria blue were found to be equally

useful. This method applies to all types of malignancy. Hodgkin's disease and various forms of leucemia give consistently positive results.

Immunochemical studies on egg white proteins. L. R. WETTER, M. COHN and H. F. DEUTSCH (introduced by A. M. PAPPENHEIMER, JR.). *Dept. of Physiological Chem., Uni. of Wisc. Medical School and Dept. Microbiology, New York Univ., College of Medicine, New York City.* Although chicken egg white (EW) possesses at least seven electrophoretic components, only ovalbumin has been extensively characterized. Immunochemical methods were used to furnish information concerning the minor and poorly understood components of chicken EW. The curve describing the quantitative precipitin reaction between EW and rabbit antisera is extremely complex. Nevertheless, when purified preparations of ovalbumin, conalbumin, ovomucoid and lysozyme were used as precipitinogens, various portions of the curve could be ascribed to reactions between these proteins and their respective antibodies. Although ovalbumin comprises 60% of EW, when the latter was used for immunization only a small proportion of the total antibody was directed against it. Conalbumin induced greater antibody production than ovalbumin although it comprises only 16% of the total protein. A very small proportion of the total antibody was directed against lysozyme and ovomucoid. The major portion of the antibody was not directed against the above four components, which comprise approximately 95% of the total protein, but rather against the remaining 5%. Rabbit anti EW sera were used to study the cross reactions of the antigens in chicken EW with the corresponding antigens in egg whites of turkey, guinea hen, goose and duck. It was demonstrated that several other components cross react in addition to ovalbumin, especially those comprising the minor portion of chicken EW.

Anaphylactic studies in mice. A. H. WHEELER, E. BRANDON, and H. PETRENCO (introduced by REUBEN L. KAHN). *Serology Laby., Univ. Hospital, Ann Arbor, Mich.* As is well known, mice do not respond typically to anaphylactic shock. Experiments were carried out in this laboratory showing that, if mice are injected with egg albumin plus Freund's adjuvant and then challenged with the egg albumin, fatal anaphylactic shock occurs in about 60% of the animals. Generally only about 10% fatality occurs in the control animals injected with the protein alone. It was also observed that, following a challenge, abortion occurs more frequently in sensitized pregnant mice than in non-sensitized ones. This finding suggests that in mice, abortion may prove a more sensitive indicator of anaphylaxis than anaphylactic death.

Demonstration, occurrence and significance of iso-immunization against the A and B factors during pregnancy. ERNEST WITEBSKY. *Dept. of Bacteriology and Immunology, Medical School, Buffalo, N. Y.* Fifty cases of heterospecific pregnancies were examined for immune anti-A and immune anti-B isoantibodies. Their presence was demonstrated by 2 methods. One consisted in the replacement of saline solution by undiluted human serum and the second method by neutralization of the isoantibodies by means of the purified blood group specific substances followed by titration in saline solution as well as in indiluted human serum. More than half of the cases examined did not reveal any trace of isoimmunization; others in varying degrees from slight to strong antibody formation. Even in those few instances in which a potent immune antibody was found, the baby upon delivery proved to be normal. In a series of investigaions carried out independently from the 50 cases mentioned, 2 umbilical cord bloods were found whose cells proved to be sensitized when suspended in normal adult serum (9-drop method). The only clue which could be found for the cause of this phenomenon was isoimmunization against the A and B factor respectively. It was interesting to note that in both instances the Coombs test was negative in our hands. Only in one baby was there slight evidence of hemolysis and jaundice. The only case of fatal erythroblastosis presumably caused by isoimmunization against the A factor so far experienced by us was that of a baby of blood group A born to a mother belonging to the blood group B (5th child). A potent immune anti-A antibody was found in the mother's circulation. The baby's umbilical cord serum also contained an immune anti-A antibody whose strength roughly compared with the immune anti-A antibody present in the mother's serum following *invitro* neutralization with A substance. It is apparently much more difficult to neutralize immune anti-A antibodies than normal isoantibodies. By comparing the cord serum with the mother's serum the impression was gained that the baby's body acted *invivo* similar to the addition of A substance to the mother's serum in the test-tube. The titer of both sera was somewhat alike. The conclusion is therefore drawn that isoimmunization against the A and B factors is only a rare cause of erythroblastosis. The demonstration of increasing antibody titer or even the presence of immune antibodies in the mother's serum is insufficient evidence to predict erythroblastotic changes in the newborn.

Relationship of blood sugar and hypoproteinemia to antibody response in diabetics. MICHAEL G. WOHL, S. O. WAIFE, STANLEY GREEN, and GEORGE B. CLOUGH (introduced by MAX B. LURIE). *Philadelphia General Hospital, Philadelphia, Penna.* Diabetic patients have a lessened capacity to form antibodies. The low antibody response to antigenic stimulation appears to bear

relationship to the blood protein values rather than to fasting blood sugar levels. Sixty-four diabetic patients were studied chemically and immunologically. In 14 patients of this series nitrogen balance studies were performed for a period of 36–52 days. Nineteen hypoproteinemic diabetic patients received protein supplementation in the form of lactalbumin hydrolysate or casein concentrate. The agglutinin response to typhoid vaccine was determined by the macroscopic method; in many of the patients a quantitative estimation of agglutinin by the micro Kjeldahl method was made. Our studies indicate that there is no relationship between the fasting blood sugar levels and the serum antibody content. On the other hand, diabetic patients with hypoproteinemia show a lower average agglutination titre than diabetics with normal blood protein values. The addition of extra protein to the diet enhances the antibody producing capacity of the hypoproteinemic diabetic patients. The protein supplementation does not appear to have an adverse effect on the course of the disease as judged by the blood sugar, CO_2 combining power of blood plasma or insulin requirement. It is noteworthy that there is no apparent relation between the degree of nitrogen storage and antibody response, indicating that the retention of protein (as N) was not immediately reflected in an increase of antibody formation.

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY

Thirty-third Annual Meeting

DETROIT, MICHIGAN, APRIL 18–22, 1949

GENERAL INFORMATION

The 1949 scientific sessions of the Federation (except motion pictures) will be held in the Masonic Temple, located at Second Boulevard and Temple Street, Detroit. To provide for the presentation of all papers accepted by the constituent Societies fourteen session rooms in the Temple have been secured. All of these rooms are conveniently reached from the Second Boulevard entrance and the Cathedral foyer.

The only *entrance to the Masonic Temple for the daytime Federation sessions* will be on *Second Boulevard* near the corner of Temple Street: the Auditorium entrance for the Federation Joint Session will be available for use on Tuesday evening only.

Registration will be located in the Masonic Temple in the Cathedral foyer, near the Second Boulevard entrance. The registration fee has been set at $5.00, and the official badge given with registration will be necessary for admittance to any session. Registration will be open on Monday from 10:00 A.M. to 10:00 P.M., and daily thereafter. Members and guests are urged to register on Monday if possible, to avoid the congestion on Tuesday morning.

Ladies who are present as guests and who are not attending sessions may register without charge at a separate registration desk in the Cathedral foyer. A program of activities has been prepared by the Ladies Entertainment Committee. Full information can be had at this desk.

Programs, abstracts and tickets to various special functions will be on sale at desks in the Cathedral foyer. An information desk will be located in the foyer of the basement-mezzanine floor.

Technical exhibits will be located in the Oak Room and the Crystal Ballroom of the Masonic Temple, on the basement floor directly below the Cathedral foyer. Members and guests will find much of interest to them in the exhibits.

Transportation between the two headquarters hotels and the Masonic Temple can be had in special buses from 8:00 to 9:15 A.M. and from 4:00 to 5:30 P.M. each day beginning Tuesday.

The Placement Service of the Federation will maintain an office in Supper Room 1 of the Masonic Temple, Tuesday, Wednesday and Thursday, from 10:00 A.M. to 4:30 P.M. Appointments between employers and applicants for positions may be made there. Applicants are requested to prepare several copies of a typed statement of vitae, including age, marital status, educational, academic and research backgrounds, references and publications, and to send a copy in advance to the Federation Secretary, 2101 Constitution Avenue, Washington 25, D. C.

Headquarters Hotels: The Book-Cadillac Hotel is headquarters for the Society of Biological Chemists, the Society for Experimental Pathology, and the Association of Immunologists. The Statler Hotel is headquarters for the American Physiological Society, the Society for Pharmacology and Experimental Therapeutics, and the Institute of Nutrition.

The Joint Session of the Federation will be held in the Auditorium of the Masonic Temple, Tuesday evening, April 19, at 8:00 P.M. *Motion pictures* will be shown Thursday evening, April 21, at 7:00 P.M., in the ball room of the Detroit-Leland Hotel.

An informal mixer will be held in the Ballrooms of the Hotel Statler on Wednesday evening, April 20, from 9:30 P.M. Refreshments will be served and an orchestra will provide music for dancing. Admission will be by ticket, given at the time of registration.

Special functions: A list of special functions, group dinners and meetings, and their locations, will be found on pages 496 and 497 of this program.

FEDERATION PROGRAM

JOINT SESSION OF THE FEDERATION

TUESDAY, APRIL 19, 8:00 P.M.

MASONIC TEMPLE AUDITORIUM

CHAIRMAN: H. P. SMITH

1. Amino acid metabolism in mutant strains of microorganisms. E. L. TATUM. *School of Biological Sciences, Stanford University, Calif.*
2. Viral multiplication. FRANK L. HORSFALL, JR. *Hospital of the Rockefeller Institute for Medical Research, New York City.*
3. The physiology of the individual—the particular and the general in quantitative biology. ANCEL KEYS. *Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis.*
4. A commentary on electronmicroscopy in the analysis of tissue structure. FRANCIS O. SCHMITT. *Department Biology, Massachusetts Institute of Technology, Cambridge, Mass.*

MOTION PICTURES

THURSDAY, APRIL 21, 7:00 P.M. — DETROIT—LELAND HOTEL BALLROOM

1. Methods of producing travelling contraction nodes in adult frog muscle fibers. *B. A. Cookson and Floyd Wiercinski (introduced by L. V. Heilbrunn). Dept. Zoology, Univ. of Pennsylvania, and Dept. Physiology, Hahnemann Medical School, Philadelphia* (Physiol.).
2. A respirator and perfusion pump with new features and an improved constant injection apparatus. *W. B. Youmans, Dept. Physiology, Univ. of Oregon Medical School, Portland* (Physiol.).
3. Action of the cardiac, pulmonic and aortic valves of the beating heart. *H. L. Smith,* Hiram E. Essex and E. J. Baldes. The Mayo Clinic and Mayo Foundation, Rochester, Minn.* (Physiol.).
4. Cholinergic nature of the vestibular receptor mechanism; forced circling movements. *H. E. Himwich, C. F. Essig,* J. L. Hampson,* P. D. Bales* and A. M. Freedman.* Medical Division, Army Chemical Center, Md.* (Physiol.).
5. Ataxia and loss of equilibrium in manganese-deficient rats. *Robert M. Hill, Dorsey E. Holtkamp,* A. R. Buchanan* and Enid K. Rutledge.* Depts. Biochemistry, Anatomy and Pathology, Univ. of Colorado Medical Center, Denver* (Biochem.).
6. Heart sounds—electrostephone and recording machine. *Harry Kirschbaum (introduced by F. F. Yonkman). Detroit* (Pharmacol.).
7. Cinéfluorography of the normal human heart. *R. F. Rushmer (introduced by T. C. Ruch). Dept. Physiology and Biophysics, Univ. of Washington, Seattle* (Physiol.).
8. Action of dilute trypsin on muscle protoplasm. *Floyd J. Wiercinski and Brian E. Cookson (introduced by John C. Scott). Dept. Physiology, Hahnemann Medical College, and Dept. Zoology, Univ. of Pennsylvania, Philadelphia* (Physiol.).
9. Artificial insemination of rabbits and transplantation of rabbit eggs. *M. C. Chang and G. Pincus. Worcester Foundation for Exptl. Biology, Shrewsbury, Mass., and Tufts College of Medicine, Boston* (Physiol.).
10. Function of the ear in health and disease. *H. G. Kobrak, Joseph E. Hind* and Robert B. Miller.* Univ. of Chicago* (Physiol.).
11. Inside the cell: enzymes in intracellular chemistry. *Alex B. Novikoff (introduced by F. J. Sichel). Dept. Pathology, Univ. of Vermont College of Medicine, Burlington* (Physiol.).

FEDERATION "MIXER"

WEDNESDAY, APRIL 20, 9:30 P.M. — HOTEL STATLER BALLROOM
Refreshments—Dancing — *Admission by Ticket*

THE AMERICAN PHYSIOLOGICAL SOCIETY

Fifty-eighth Annual Meeting

An asterisk following an author's name denotes "by invitation".

TUESDAY, APRIL 19, 9:00 A.M.

Respiration

FOUNTAIN BALLROOM

1. Effects of unequal blood flow and ventilation on the alveolar-arterial gradient. *Hermann Rahn. Dept. Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, N. Y.*
2. Attempt at direct measurement of values required for calculating the pulmonary diffusion coefficient for oxygen. *Christian J. Lambertsen, John K. Clark,* Domingo M. Aviado, Jr.,* Robert G. Pontius,* Earl S. Barker* and John Moyer.* Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*
3. Mass spectrographic studies on expired and alveolar air. *George H. Kydd III* and F. A. Hitchcock. Dept. Physiology, Ohio State Univ., Columbus.*
4. Analysis of ventilation-perfusion relationships in the lungs. *R. L. Riley and A. Cournand. Dept. Medicine, Columbia Univ. College of Physicians and Surgeons, New York City.*
5. Oximeter measurements in pulmonary and cardiac disease. *J. K. W. Ferguson, D. M. Finlayson and I. M. Hilliard. Dept. Pharmacology, Univ. of Toronto, and Toronto Western Hospital, Ontario, Canada.*
6. 'Saturation tension' and alveolar-arterial pO_2 gradient as expressions of 'venous admixture' in the lungs. *J. C. Douglas,* R. L. Riley and A. Cournand. Dept. Physiology, Univ. of Western Ontario Medical School, London, Ontario, and Dept. Medicine, Columbia Univ. College of Physicians and Surgeons, New York City.*
7. Evaluation of methods for determining alveolar gas tensions in man. *Earl S. Barker,* Robert G. Pontius,* Domingo M. Aviado, Jr.* and Christian J. Lambertsen. Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*
8. The CO_2 pressure gradient across the placenta. *Donald H. Barron. Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*
9. A method for collecting end-expiratory air from the oral cavity. *Gerson Lesser, Morton Galdston and Marylin Press (introduced by J. Murray Steele). Research Service, Third New York Univ. Medical Div., and Goldwater Memorial Hospital, New York City.*
10. Direct determination of gas tensions by blood. *Albert Roos and H. Black (introduced by H. L. White). Lab. of Applied Thoracic Physiology, and Dept. Physiology, Washington Univ. School of Medicine, St. Louis.*
11. Limitations of the polarographic method of determining the oxygen tension in whole blood. *J. Percy Baumberger, Gabor Markus* and Kathleen Bardwell.* Dept. Physiology, Stanford Univ. School of Medicine, Calif.*
12. Oxidation of CO to CO_2 by the intact animal. *Robert T. Clark, Jr., J. Newell Stannard and W. O. Fenn. Depts. Physiology, Vital Economics and Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, N. Y.*

TUESDAY, APRIL 19, 9:00 A.M.

Heart

CONSISTORY PARLOR

1. Contractile force of the heart in open-chest mammalian experiments. *H. H. Brill, M. deV. Cotton and R. P. Walton (introduced by T. G. Bernthal). Dept. Pharmacology, Medical College of South Carolina, Charleston.*
2. Effect of epinephrine on the water and electrolyte composition of cardiac muscle. *Wm. v. B. Robertson and Pincus Peyser (introduced by F. J. Sichel). Division Exptl. Medicine and Dept. Biochemistry, Univ. of Vermont College of Medicine, Burlington.*
3. Influence of adrenaline on the cardiodepressor effect of acetylcholine and the vagus nerve. *S. Middleton and J. Talesnik (introduced by Harold C. Wiggers). Physiological Institute, Univ. of Chile, Santiago.*
4. Accuracy of pressures recorded through a catheter. *W. H. Pritchard,* R. W. Eckstein, R. Eckel,* C. L. Parsons* and T. E. Lowe.* Dept. Medicine, Western Reserve Univ., Cleveland.*
5. Effect of intrapulmonic and intrathoracic pressure variations on left atrial pressure. *D. F. Opdyke and G. A. Brecher.* Dept. Physiology, Western Reserve Univ. Medical School, Cleveland.*

6. Measurement of cardiac volume in the dog from x-ray shadow area. *W. F. Hamilton, Jr.* and Philip Dow. Depts. Physiology and Roentgenology, Univ. of Georgia School of Medicine, Augusta.*
7. Late development of ectopic ventricular activity following coronary occlusion. *A. Sidney Harris and Robert H. Kokernot.* Dept. Physiology, Baylor Univ. College of Medicine, Houston, Tex.*
8. Effect of dibenamine on ventricular fibrillation following sudden coronary occlusion. *G. W. Manning. RCAF Institute of Aviation Medicine, Toronto, and Dept. Physiology, Univ. of Western Ontario, London, Ontario, Canada.*
9. Use of intravenous quinidine orally in certain mechanism disorders. *John W. Chriss and Milton R. Hejtmancik (introduced by George R. Herrmann). Univ. of Texas Medical Branch, Galveston.*
10. A constant delivery pipette for analysis of small volumes. *R. C. Deering (introduced by H. C. Bazett). Dept. Physiology, Univ. of Pennsylvania Medical School, Philadelphia.*
11. A small plastic tubing technique for right and left heart catheterization. *H. F. Fitzpatrick, T. G. Schnabel, Jr. and L. H. Peterson (introduced by H. C. Bazett). Dept. Physiology, Univ. of Pennsylvania Medical School, Philadelphia.*
12. Indirect estimation of cardiac output in man from carbon dioxide exchange. *Cyril A. Forssander,* Herbert Kramer,* C. J. Martin,* Colin White* and H. C. Bazett. Dept. Physiology, Univ. of Pennsylvania Medical School, Pennsylvania.*

TUESDAY, APRIL 19, 9:00 A.M.

Adrenals

ROOM 4

1. Corticosteroid release from isolated adrenal gland. *Oscar Hechter. Worcester Foundation Exptl. Biology, Shrewsbury, Mass.*
2. Secretion of cortical hormone by the isolated adrenal. *Marthe Vogt (introduced by H. B. van Dyke). Pharmacological Laboratory, Univ. New Buildings, Edinburgh, Scotland* (Pharmacol.).
3. Fat-mobilizing hormone of the anterior pituitary. *Richard W. Payne (introduced by E. B. Astwood). Joseph H. Pratt Diagnostic Hospital and Dept. Medicine, Tufts Medical School, Boston.*
4. Pituitary adrenocorticotropin and plasma hypertensinogen concentration of dogs. *F. W. Haynes, P. H. Forsham,* G. W. Thorn, and L. Dexter.* Dept. Medicine, Harvard Medical School, and Medical Clinic, Peter Bent Brigham Hospital, Boston.*
5. Antagonism of adrenocorticotrophic hormone (ACTH) to desoxycorticosterone acetate (DCA) on electroshock threshold and electrolytes. *Dixon M. Woodbury,* Chi-Ping Cheng* and George Sayers. Depts. Physiology and Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*
6. Effect of desoxycorticosterone acetate (DCA) on pituitary content of adrenocorticotrophic hormone (ACTH) after adrenalectomy. *Chi-Ping Cheng,* Marion A. Sayers* and George Sayers. Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*
7. Role of the adrenal cortex in the abnormal insulin and adrenaline response of hypophysectomized dogs. *R. C. de Bodo, S. P. Kiang* and I. H. Slater.* Dept. Pharmacology, New York Univ. College of Medicine, New York City.*
8. The histo-physiology of the adrenal cortex. *Hans Selye and Helen Stone.* Institut Médecine et Chirurgie expérimentales, Univ. Montréal, Canada.*
9. Adrenal cortical hormone levels in blood following 'alarming stimuli'. *K. E. Paschkis, A. Cantarow and D. Boyle. Jefferson Medical College, Philadelphia.*
10. Effects of cold environment and of muscular work on adrenal function. *A. Desmarais (introduced by L. P. Dugal). Dept. d'Acclimatation, Institut de Biologie Humaine, Laval Univ., Quebec, Canada.*
11. Adrenocorticotropin and human chronic lymphatic leukemia. *Leon Hellman (introduced by R. E. Weston). Medical Division, Montefiore Hospital, New York City.*
12. Relation of adrenal cortex to increased macrophagic activity induced by starvation. *Albert S. Gordon and Grace F. Katsh.* Dept. Biology, Washington Square College of Arts and Sciences, New York Univ., New York City.*
13. Relation of adrenal and spleen in regulation of the leucocyte picture of the rat. *Abraham Dury and Habeeb Bacchus.* Dept. Physiology George Washington Univ. School of Medicine, Washington, D. C.*

TUESDAY, APRIL 19, 9:00 A.M.

Neuro-Physiology

PARLOR F

1. Electrical and functional activity of spinal motor neurons. *John Hunter, Richard Lillie* and Robert Gesell. Dept. Physiology, Univ. of Michigan, Ann Arbor.*

2. 'Impedance' variation in the nerve membrane during passage of the natural action potential. *Otto H. Schmitt. Depts. Zoology and Physics, Univ. of Minnesota, Minneapolis.*
3. Alteration of neuron excitability by axon section. *Berry Campbell, Edgar Gasteiger and Vernon H. Mark. Dept. Anatomy, Univ. of Minnesota, Minneapolis.*
4. Exchange of ions across nerve membranes. *Mortimer A. Rothenberg (introduced by David Nachmansohn). Dept. Neurology, Columbia Univ. College of Physicians and Surgeons, New York City.*
5. Electrical phenomena in the squid giant axon. *Abraham M. Shanes. Dept. Physiology and Biophysics, Georgetown Univ. School of Medicine, Washington, D. C., and Marine Biological Lab., Woods Hole, Mass.*
6. Effects of anticonvulsants on properties of frog sciatic nerve. *J. E. P. Toman, M. D. Greenhalgh,* J. S. Carlson,* and G. H. Bjorkman.* Depts. Physiology and Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*
7. Action potentials of giant fibers of the crayfish. *C. A. G. Wiersma. Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena.*
8. A phase-boundary mechanism for impulse formation, transmission and synaptic properties of nerve. *R. Beutner and T. C. Barnes. Dept. Pharmacology, Hahnemann Medical College and Hospital of Philadelphia.*
9. Cholinergic nature of vestibular receptor mechanism; forced circling movements. *C. F. Essig,* J. L. Hampson,* P. D. Bales* and H. E. Himwich. Medical Division, Army Chemical Center, Md.*
10. Summation of stimuli studied with the aid of anticholinesterases. *Jeane Siskel Frey* and Robert Gesell. Dept. Physiology, Univ. of Michigan, Ann Arbor.*
11. Variation of choline acetylase content of brain in stressed and unstressed normal and adrenalectomized rats. *Ruven Greenberg (introduced by F. A. Hartman). U. S. Public Health Service Fellow, Dept. Physiology, Ohio State Univ., Columbus.*
12. Effects of di-isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity. *J. L. Hampson,* C. F. Essig,* Alice Willis* and H. E. Himwich. Medical Division, Army Chemical Center, Md.*

TUESDAY, APRIL 19, 9:00 A.M.

General Physiology

MURAL ROOM

1. Colchicine derivatives: toxicity and antimitotic effect. *W. Fleischmann, A. Goldin,* B. Goldberg,* L. G. Ortega,* A. D. Bergner* and G. E. Ullyot.* Medical Division, Army Chemical Center, Md.; Dept. Preventive Medicine, Johns Hopkins Univ. School of Medicine, Baltimore, Md., and Smith, Kline and French Laboratories, Philadelphia.*
2. Production of adeno-carcinoma of breast in Wistar rats by gastric instillation of methylcholanthrene. *Harry Shay, Ernest A. Aegerter,* Margot Gruenstein* and S. A. Komarov. Samuel S. Fels Research Institute and Dept. Pathology, Temple Univ. School of Medicine, Philadelphia.*
3. Influence of hydrostatic pressure and of urethan on the thermal inactivation rate of bacteriophage. *Frank H. Johnson and Ruth A. C. Foster.* Biological Lab., Princeton Univ., N. J.*
4. Volumetric Apparatus for Studies of Tissue Metabolism. *Reidar Wennesland (introduced by J. Field). Arctic Research Laboratory, Point Barrow, Alaska, and Dept. Physiology, Stanford Univ., Calif.*
5. Comparison of a volumetric respirometer with the Warburg method in measurement of brain respiration. *C. N. Peiss and R. Wennesland (both introduced by J. Field). O.N.R. Arctic Research Laboratory, Point Barrow, Alaska, and Dept. Physiology, Stanford Univ., Calif.*
6. Effects of homologous carbamates on the respiration of blocked and active embryonic cells. *Joseph H. Bodine and Laurence R. Fitzgerald.* Zoology Lab., State Univ. of Iowa, Iowa City.*
7. Conditions leading to aerobic formation of VDM by liver. *Robert F. Furchgott,* Benjamin W. Zweifach and Ephraim Shorr. Dept. Medicine, Cornell Univ. Medical College and New York Hospital, New York City* (Biochem.).
8. Adenosine phosphorylation by rabbit kidney homogenate. *David Rapport, Attilio Canzanelli and Ruth Guild.* Dept. Physiology, Tufts College Medical School, Boston.*
9. Oxygen consumption of liver slices from febrile and normal rabbits. *V. E. Hall, J. Fishgold* and R. Grant.* Stanford Univ., Calif.*
10. Oxygen consumption of the descending aortae of adrenalectomized rats in the presence of various substrates. *Phyllis M. Arscott* and Clifford A. Angerer. Dept. Physiology, Ohio State Univ., Columbus.*
11. Effects of an anterior pituitary extract on the metabolism of the liver. *James Campbell and I. W. F. Davidson.* Dept. Physiology, Univ. of Toronto, Canada.*
12. 'In vitro' metabolism of bone marrow in ringer-bicarbonate medium containing no glucose. *John D. Evans and Robert M. Bird*

B & Z Library

(*introduced by Eugene F. Du Bois*). *Dept. Physiology, Cornell Univ. Medical College, New York City.*

13. Effect of pteroylglutamic acid and 4-amino pteroylglutamic acid on the growth of sarcoma 180. *A. Goldin,* B. Goldberg,* L. G. Ortega* and E. B. Schoenbach. Medical Division, Army Chemical Center, Md., and Dept. Preventive Medicine, Johns Hopkins Univ. School of Medicine, Baltimore.*

TUESDAY, APRIL 19, 1:30 P.M.

Respiration

FOUNTAIN BALLROOM

1. Nature of breathing impairment in anthracosilicosis. *Hurley L. Motley and Leonard P. Lang.* Dept. Medicine, Jefferson Medical College, and Cardio-Respiratory Laboratory, Barton Memorial Division of Jefferson Hospital, Philadelphia.*
2. Elasticity of the rat lung. *Richard W. Lawton and Allen L. King (introduced by James D. Hardy). Dept. Physiology, Cornell Univ. Medical College, New York City, and Dept. Physics, Dartmouth College, Hanover, N. H.*
3. Resuscitation from fresh water drowning. *David C. Fainer,* Clement G. Martin,* Henry Schwerma and A. C. Ivy. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
4. Nature of apneusis and the pontine influence on respiration. *Hebbel E. Hoff and C. G. Breckenridge.* Dept. Physiology, Baylor Univ. College of Medicine, Houston.*
5. Effect of spinal cord transection on ventilatory response to exercise. *Donald P. Morgan* and Fred S. Grodins. Dept. Physiology, Northwestern Univ. Medical School, Chicago.*
6. Effect of spinal cord transection on blood acid-base balance during exercise. *Fred S. Grodins and Donald P. Morgan.* Dept. Physiology, Northwestern Univ. Medical School, Chicago.*
7. Effect of position on respiratory rate of anesthetized dogs. *E. A. Reed and J. C. Scott. Dept. Physiology, Hahnemann Medical College, Philadelphia.*
8. Application of Gray's theory of respiratory control to the hyperpnea produced by 'passive' exercise. *Arthur B. Otis. Dept. Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, N. Y.*
9. Alterations in reflex muscular contractions induced by positive intrapulmonic pressure. *Leonard H. Elwell* and John W. Bean. Dept. Physiology, Univ. of Michigan, Ann Arbor.*
10. Pressure responses of cerebrospinal fluid to diffusion respiration and carbon dioxide inhalation. *Joseph N. Spencer,* Eli S. Goldensohn,* Richard W. Whitehead, Robert F. Grover* and William B. Draper. Dept. Physiology and Pharmacology, Univ. of Colorado Medical Center, Denver.*
11. The spleen as a blood reservoir during hypoxia. *Kurt Kramer and Ulrich C. Luft (introduced by Harry F. Adler). Dept. Physiology, USAF School of Aviation Medicine, Randolph Field, Tex.*
12. Time course of failure of the visual pathway in rabbits during anoxia. *Werner K. Noell and Herman I. Chinn (introduced by Harry F. Adler). USAF School of Aviation Medicine, Randolph Field, Tex.*

TUESDAY, APRIL 19, 1:30 P.M.

Heart

CONSISTORY PARLOR

1. Determination of cardiac output by an improved electrical conductivity method. *Raymond C. Ingraham. Dept. Physiology, Univ. of Illinois, College of Medicine, Chicago.*
2. Oximetric method for determination of the oxygen saturation of mixed venous blood in man. *Julian Knutson,* Bowen E. Taylor* and Earl H. Wood. Section on Physiology, Mayo Foundation, Univ. of Minnesota, Rochester.*
3. Variability and reliability of cardiac index estimations made with the high-frequency, undamped ballistocardiograph. *Robert M. Paine* and Nathan W. Shock. Section on Cardiovascular Diseases and Gerontology, National Heart Institute, Bethesda, Md., and the Baltimore City Hospitals.*
4. Relation between ballistocardiograms and systolic velocity curves. *Isaac Starr, R. L. Mayock* and O. Horwitz.* Depts. Therapeutic Research and Medicine, Univ. of Pennsylvania, Philadelphia.*
5. Kinetic and potential energy of the work of the human heart. *O. Prec,* L. N. Katz, R. Rosenman* and L. W. Sennett.* Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago.*
6. Measurements of heart output with the electrokymograph. *Gordon C. Ring, M. J. Oppenheimer and Miriam Balaban.* Temple Univ. Medical School, Philadelphia.*
7. The capacitance method for the study of cardiac activity. *William V. Whitehorn and Edward R. Perl.* Dept. Physiology, Univ. of Illinois College of Medicine, Chicago.*

8. Production of constrictive pericarditis in dogs. *A. P. Fishman, L. H. Rubenstein, L. W. Sennett and K. Kuramoto (introduced by L. N. Katz). Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago.*
9. Vasotropic principles in blood (VEM and VDM) and renal hemodynamics in chronic heart failure. *R. Mokotoff,* D. J. W. Escher,* I. S. Edelman,* J. Grossman,* L. Leiter,* R. E. Weston, B. W. Zweifach and E. Shorr. Medical Division, Montefiore Hospital, and Dept. Medicine, Cornell Univ. Medical College, New York Hospital, New York City.*
10. Measurement of renal venous pressure in normal man and in patients with congestive heart failure. *M. Harrison Maxwell, Ernest S. Breed and Irving L. Schwartz (introduced by Homer W. Smith). Dept. Physiology, New York Univ. College of Medicine, New York City.*
11. Effect of theophylline ethylene diamine on renal function in control subjects and decompensated cardiac patients. *James O. Davis* and Nathan W. Shock. Section on Cardiovascular Diseases and Gerontology, National Heart Institute, National Institutes of Health, Bethesda, Md., and the Baltimore City Hospitals.*
2. Femoral arterial pulse in aortic insufficiency. *Robert S. Alexander. Dept. Physiology, Western Reserve Univ. Medical School, Cleveland.*

TUESDAY, APRIL 19, 1:30 P.M.

Adrenals

ROOM 4

1. Thiocyanate and mannitol space and native ionic balance in acute adrenal insufficiency. *James B. Flanagan* and R. R. Overman. Division Physiology, Univ. of Tennessee College of Medicine, Knoxville.*
2. Effect of salt-sugar solution and DCA on lactation. *Thomas C. Smith (introduced by F. L. Hisaw). Biological Labs., Harvard Univ., Cambridge.*
3. Effects of DOCA and adrenal cortical extracts on survival of adrenalectomized rats after burning. *S. S. You* and E. A. Sellers. Dept. Physiology, Univ. of Toronto, Canada.*
4. Effect of vitamine deficiency, thyroid and adrenal hormones on oxidation enzymes of rat tissues. *Samuel R. Tipton and Frances M. Colvin.* Dept. Zoology and Entomology, Univ. of Tennessee, Knoxville.*
5. Effects of steroids on endocrine system of the starved guinea pig. *Savino A. D'Angelo, Albert S. Gordon and Harry A. Charipper. Dept. Biology, Washington Square College Arts and Science, New York Univ., New York City.*
6. Fluorometric method of determination of adrenalin in blood. *William W. Shingleton and H. M. Baker (introduced by K. S. Grimson). Dept. Surgery, Duke Univ., Durham.*
7. Role of the adrenal cortex in the excretion of purines. *Meyer Friedman, Donald Bernstein* and Sanford O. Byers.* Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital, San Francisco.*
8. Experimental studies in ascorbic acid metabolism. *Walter M. Booker, Raymond Hayes* and Marianna Sewell.* Dept. Pharmacology, College of Medicine, and Dept. Oral Medicine, College of Dentistry, Howard Univ., Washington, D. C.*
9. Mode of action of parathormone. *Philip Handler, W. J. A. DeMaria* and D. V. Cohn.* Depts. Biochemistry and Pediatrics, Duke Univ. School of Medicine, Durham.* (Biochem.)
10. Metabolism of rat testis *in vitro*. *Jay Tepperman and Helen M. Tepperman.* Dept. Pharmacology, Syracuse Univ. College of Medicine, N. Y.*
11. Connective tissue and parenchymal changes in ovary of the rat. *H. R. Catchpole and S. C. Pan.* Dept. Pathology, Univ. of Illinois College of Medicine, Chicago.*
12. Lipide studies on whole anterior lobe, nuclei, and cytoplasmic particulates of lamb pituitary glands. *M. H. Hack (introduced by Hubert R. Catchpole). Dept. Pathology, Univ. of Illinois College of Medicine, Chicago.*
13. Chromogenic steroid occurring in the urine of males after muscular exertion. *H. S. Guterman and L. M. Kraus (introduced by R. Levine). Dept. Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago.*

TUESDAY, APRIL 19, 1:30 P.M.

Neuro-Physiology

PARLOR F

1. Determination of acetylcholine in cell-free enzyme systems by a colorimetric method. *Emily F. Hedal and Shlomo Hestrin (introduced by David Nachmansohn). Dept. Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.*
2. Mechanism of toxic effects of inhibitors of acetylcholine-esterase. *David Nachmansohn.*

Dept. Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.

3. Effects of eserine and pilocarpine on the parasympathetically denervated iris of the cat. *Enid A. Neidle (introduced by Walter S. Root). Dept. Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.*
4. DFP and reflex activity in salamander larvae. *Charles H. Sawyer (introduced by J. E. Markee). Dept. Anatomy, Duke Univ., Durham.*
5. Effect on action potential of nerve and muscle of 2-methyl naphthoquinone. *Clara Torda and Harold G. Wolff. New York Hospital and Depts. Medicine (Neurology) and Psychiatry, Cornell Univ. Medical College, New York City.*
6. Chemical structure and physiological properties of acetylcholine. *John H. Welsh. Biological Labs., Harvard Univ., Cambridge.*
7. Neural mechanisms involved in itching. *David T. Graham and Helen Goodell (introduced by Harold G. Wolff). New York Hospital and Depts. Medicine (Neurology) and Psychiatry, Cornell Univ. Medical College, New York City.*
8. Action of neurohumoral agents in the human skin. *M. I. Grossman, Henry D. Janowitz* and R. R. Sonnenschein.* Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
9. Thalamic representation of tactile sensibility in the Macaque monkey. *Vernon Mountcastle and Elwood Henneman (introduced by Philip Bard). Dept. Physiology, Johns Hopkins Univ. School of Medicine, Baltimore.*
11. Response of individual Pacinian corpuscles to external stimulation. *Donald Scott, Jr. (introduced by Frank Brink, Jr.). Johnson Foundation, Univ. of Pennsylvania, Philadelphia.*
12. Reorganization of sensory function in amputation stumps: two-point discrimination. *Hans-Lukas Teuber,* Howard P. Krieger* and Morris B. Bender. Psychophysiological Lab., Dept. Neurology, New York Univ. College of Medicine, New York City.*

TUESDAY, APRIL 19, 1:30 P.M.

General Physiology

MURAL ROOM

1. Nitrogenous excretion in colpidium campylum. *Roland M. Nardone (introduced by Charles G. Wilber). Biological Laboratory Fordham Univ., New York City.*
2. Metabolism in colpidium campylum. *Gerald R. Seaman (introduced by Charles G. Wilber). Biological Laboratory, Fordham Univ., New York City.*
3. Action of toxins of Streptococcus pyogenes, on cell respiration. *F. R. Hunter (introduced by A. K. Parpart). Dept. of Zoological Sciences, Univ. of Oklahoma, Norman.*
4. Tissue respiration in the polar cod (Boreogadus saida) as a function of temperature. *John Field and C. N. Peiss.* O. N. R. Arctic Research Laboratory, Point Barrow, Alaska and Dept. Physiology, Stanford Univ., Calif.*
5. Enzymes in the grasshopper egg. *Laurence R. Fitzgerald (introduced by J. H. Bodine). Zoological Laboratories, State Univ. of Iowa, Iowa City.*
6. Time-dose study of biochemical responses of rats to x-radiation. *N. North* and L. F. Nims. Brookhaven National Laboratory, Upton, L. I., N. Y.*
7. Effects of ionizing radiation on anaerobic CO_2 production and colony production in Saccharomyces cerevisiae. *Frederick G. Sherman and Herman B. Chase (introduced by P. H. Mitchell). Brown Univ., Providence.* (Biochem.)
8. Peripheral vascular reactions in the wing of the bat after total-body x-irradiation. *Douglas E. Smith, George Svihla* and Harvey M. Patt. Argonne National Laboratory, Chicago.*
9. Analytic study of effects of various patterns of x-irradiation on survival. *George A. Sacher, John Sackis* and Austin M. Brues. Argonne National Laboratory, Chicago.*
10. Relation between growth and O_2 consumption as affected by x-irradiation in Serratia marcescens. *Ann C. Fowle* and Kenneth C. Fisher. Univ. of Toronto, Canada.*
11. Effects of x-radiation on water and electrolyte metabolism in the rat. *Abraham Edelmann. Brookhaven National Laboratory, Upton, L. I., N. Y.*
12. Experimental production of femoral fractures. *F. Gaynor Evans, Herbert E. Pedersen, John F. Hayes and Herbert R. Lissner (introduced by M. Mason Guest). Depts. Anatomy and Engineering Mechanics, Wayne Univ., Detroit.*

TUESDAY, APRIL 19, 4:30 P.M.

Physiology Business Meeting

FOUNTAIN BALLROOM

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

Program on page 418

WEDNESDAY, APRIL 20, 9:00 A.M.

Kidney

FOUNTAIN BALLROOM

1. Non-utilization of mannitol in uremic patients. *J. Russell Elkinton. Dept. Internal Medicine, Yale Univ. School of Medicine, New Haven, Conn.*
2. Determination of filtration rate and renal plasma flow in dogs without analysis of urine. *C. Riley Houck. Division Physiology, Univ. of Tennessee, Memphis.*
3. Modified procedure for inulin determination in plasma and urine. *J. Maxwell Little, June Harris* and Lewis Franklin.* Dept. Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.*
4. Determination of creatinine in blood and urine. *Ruth S. Hare* and Kendrick Hare. Dept. Pediatrics, New York Hospital and Cornell Univ. Medical College, New York City.*
5. Renal excretion of creatinine in man. *Kendrick Hare, Harold Goldstein*, Henry L. Barnett,* Helen McNamara* and Ruth S. Hare.* Dept. Pediatrics, New York Hospital and Cornell Univ. Medical College, New York City.*
6. Renal and hormonal mechanisms of cold diuresis. *Richard A. Bader, J. W. Eliot, and David E. Bass (introduced by H. S. Belding) QMC Climatic Research Laboratory, Lawrence, Mass.*
7. Blood changes associated with cold diuresis. *John W. Eliot, Richard A. Bader and David E. Bass (introduced by H. S. Belding). QMC Climatic Research Laboratory, Lawrence, Mass.*
8. Thiomerin— a subcutaneously injectable mercurial diuretic. *J. Grossman,* R. E. Weston, I. S. Edelman* and L. Leiter. Medical Division, Montefiore Hospital, New York City.*
9. Renal dynamics and salt and water excretion in the dog following expansion of extracellular fluid. *Laurence G. Wesson, Jr., W. Parker Anslow, Jr., Lawrence G. Raisz, Alfred A. Bolomey and Michael Ladd (introduced by Homer W. Smith). Dept. Physiology, New York Univ. College of Medicine, New York City.*
10. Effects of mercurial diuresis on glucose reabsorption. *R. E. Weston, J. Grossman,* I. S. Edelman,* D. J. W. Escher,* L. Leiter* and L. Hellman.* Medical Division, Montefiore Hospital, New York City.*
11. Diuretic effects of neutral and alkaline Na salts in hypoproteinemic edema. *Samuel A. Corson and Elizabeth O'Leary.* Dept. Physiology, Howard Univ. School of Medicine, Washington, D. C.*
12. Antidiuretic action of crystalline VDM (ferritin, apoferritin). *Silvio Baez,* Abraham Mazur and Ephraim Shorr. Dept. Medicine, Cornell Univ. Medical College and New York Hospital, New York City.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Heart

CONSISTORY PARLOR

1. Experimental mitral insufficiency. *W. E. Adams, E. B. Bay and M. M. Newman (introduced by Lester R. Dragstedt). Depts. Medicine and Surgery, Univ. of Chicago.*
2. Effect of moderate hypoxemia, related to intracardiac venous shunts, on the systemic blood flow. *Howard B. Burchell, Bowen E. Taylor, Julian R. Knutson and Earl H. Wood. Division Medicine and Section on Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minn.*
3. Pressure changes in pulmonary artery and aorta before and after ligation of patent ductus arteriosus. *Robert F. Grover,* Henry Swann II* and Clarence A. Maaske. Depts. Physiology and Pharmacology, and Surgery, Univ. of Colorado Medical Center, Denver.*
4. Atrial hemodynamics in the presence of experimentally produced interatrial septal defects. *R. C. Little,* J. G. Hawley* and D. F. Opdyke. Dept. Physiology, Western Reserve Univ. Medical School, Cleveland.*
5. A case of congenital heart disease with cyanotic episodes. *W. F. Hamilton, J. A. Winslow* and W. F. Hamilton, Jr.* Depts. Physiology, Medicine and Roentgenology, Univ. Georgia School of Medicine, Augusta.*
6. Observations on congenital heart cases with the oxyhemograph. *Vivian G. Behrmann, Frank W. Hartman,* Robert F. Ziegler* and Conrad R. Lam.* Depts. Laboratories, Cardiology and Surgery, Henry Ford Hospital, Detroit.*
7. A new blood supply for ischemic hearts, by connecting the aorta with the coronary vessels. *Joseph Thomas Roberts. Univ. of Buffalo School of Medicine, Buffalo, N. Y.*

and Veterans Administration Hospital, Batavia, N. Y.

8. Effect of intravenous aminophylline on coronary blood oxygen. *Elwood L. Foltz, Alan J. Rubin and William A. Steiger (introduced by Carl F. Schmidt). Dept. Pharmacology, Univ. of Pennsylvania, Philadelphia.*
9. Coronary blood flow following stimulation of the cardiac accelerator nerves. *R. W. Eckstein, M. Stroud,* Charles V. Dowling,* Robert Eckel* and W. H. Pritchard.* Dept. Medicine, Western Reserve Univ., Cleveland.*
10. Effects of over and under perfusion upon coronary arterial blood flow. *R. Eckel,* R. W. Eckstein, M. Stroud* and W. H. Pritchard.* Dept. Medicine, Western Reserve Univ., Cleveland.*
11. Cardiac oxygen consumption and efficiency in man. *R. J. Bing, M. Hammond,* J. Handelsman, S. Powers* and F. Spencer.* Dept. Surgery, Johns Hopkins Univ. School of Medicine and Johns Hopkins Hospital, Baltimore.*
12. A quantitative comparison of Goldberger's augmented unipolar limb leads (aV) and Wilson's original V-leads. *Ernst Simonson and Ancel Keys. Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Gonads

ROOM 4

1. Response of the young cockerel to methyl folic acid and testosterone propionate. *M. X. Zarrow and I. B. Koretsky (introduced by F. L. Hisaw). Biological Labs., Harvard Univ., Cambridge, Mass.*
2. Role of the sertoli cell in the testis-pituitary axis in men. *Carl G. Heller, William O. Maddock,* Edwin C. Jungck* and Warren O. Nelson. Depts. Medicine and Physiology, Univ. of Oregon Medical School, Portland, and Dept. Anatomy, Univ. of Iowa Medical School, Iowa City.*
3. Vascular perfusion of the prostate gland of the dog. *Perry B. Hudson,* W. W. S. Butler, III,* Herbert Brendler* and W. W. Scott. Brady Urological Institute, Johns Hopkins Hospital, Baltimore.*
4. Potentiation of the prolactin effect. *Erwin Schwenk, Sibylle Tolksdorf, Roy McCullagh and Richard Tislow. Biological Research Laboratories and Chemical Research Division, Schering Corporation, Bloomfield, N. J.*
5. Assay of FSH, ICSH, and luteotrophin in the hypophysectomized rat: clinical applications. *William O. Maddock,* Edwin C. Jungck,* Demetrios A. Rigas* and Carl G. Heller. Depts. Physiology and Medicine, Univ. of Oregon Medical School, Portland.*
6. Adsorption of urinary gonadotrophin on kaolin. *James T. Bradbury, Elwyn Brown and Willis E. Brown. Depts. Gynecology, Univ. of Louisville, Ky., and Univ. of Iowa, Iowa City.*
7. Separation of natural estrogens by countercurrent distribution. *Lewis L. Engel,* Wilson R. Slaunwhite, Jr.,* Priscilla Carter* and Ira T. Nathanson. Mass. General Hospital and Harvard Medical School, Boston.*
8. An indication that the ovulating-hormone release-inducing action of progesterone is an indirect one. *Irving Rothchild (introduced by D. C. Smith). Bureau Animal Industry, Beltsville, Md.*
9. Bone absorption and relaxation of guinea pig pelvis by prolonged estrogenic injections. *William C. Young* and Frederick E. Emery. Dept. Physiology and Pharmacology, Univ. of Arkansas School of Medicine, Little Rock.*
10. Suppression of urinary chorionic gonadotrophin, symptoms and pulmonary metastases in chorionepithelioma by diethylstilbestrol. *Edwin C. Jungck,* William O. Maddock,* Clifford L. Fearl* and Carl G. Heller. Depts. Physiology, Gynecology and Medicine, Univ. of Oregon Medical School, Portland.*
11. Stimulating effects of light on sexual activity of the female opossum during the nonbreeding season. *Charles F. Morgan. Dept. Physiology and Biophysics, Georgetown Univ. School of Medicine, Washington, D. C.*
12. Sub-estrous response to dietary restriction and reversal in castrate C_3H mice. *Joseph T. King, Carmen B. Casas* and M. B. Visscher. Dept. Physiology, Univ. of Minnesota, Minneapolis.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Respiration—Decompression

ROOM 3

1. Effect of rest intervals on maintenance of previously induced high altitude acclimatization. *Paul D. Altland (introduced by Heinz Specht). Lab. of Physical Biology, Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.*
2. Effect of O_2 administration on the survival of dogs explosively decompressed to 30 mm. Hg. *John P. Kemph* and F. A. Hitchcock.*

Dept. Physiology, Ohio State Univ., Columbus.

3. Effect of hypoxia of 18,000 feet simulated altitude on myoglobin content of dogs. *William J. Bowen. Lab. of Physical Biology, Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.*
4. Acclimatization to histotoxic anoxia. *David W. Northrup and Ross Bell, Jr.* Dept. Physiology, West Virginia Univ. School of Medicine, Morgantown.*
5. Effect of anoxic anoxia on sheep blood sugar and hemoglobin. *Edward J. Van Liere, J. Clifford Stickney and David W. Northup. Dept. Physiology, West Virginia Univ. School of Medicine, Morgantown.*
6. Blood histamine in hypoxia. *William L. Burkhardt,* Don Flickinger* and Harry F. Adler. Dept. Physiology, USAF School of Aviation Medicine, Randolph Field, Tex.*
7. Effects of low environmental atmospheric pressure on the blood of turtles. *Louis A. Susea (introduced by Charles G. Wilber). Biological Lab., Fordham Univ., New York City.*
8. Electrocardiographic observations after explosive decompression of dogs to 30 mm. Hg. *Buford H. Burch (introduced by F. A. Hitchcock). Dept. Physiology, Ohio State Univ., Columbus.*
9. Cardiac changes in monkeys following explosive decompression to extreme altitudes. *Roy Dowling and Samuel Gelfan. Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*
10. Blood pressure changes following explosive decompression to extreme altitudes. *Attie Yvonne Werner* and Samuel Gelfan. Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*
11. Pressure changes and hemorrhage in the middle ear of monkeys resulting from decompression and recompression. *Hsiang-Tung Chang,* Rodolfo Margaria* and Samuel Gelfan. Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*
12. Conditions influencing explosive decompression injury. *E. L. Corey. Univ. of Virginia Medical School, Charlottesville.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Neuro-Physiology

PARLOR F

1. Excitability changes of single visual receptor cells following flashes of light of intensity near threshold. *I. H. Wagman, H. K. Hartline and Lorus J. Milne.* Dept. Physiology, Jefferson Medical College, and Johnson Research Foundation, Univ. of Pennsylvania, Philadelphia.*
2. Nature of cutaneous hyperalgesia. *Harold G. Wolff, H. Goodell* and James D. Hardy. New York Hospital and Depts. Medicine, Psychiatry and Physiology, Cornell Univ. Medical College, New York City.*
3. Temporal relations in the auditory pathway. *John M. Brookhart and Harlow W. Ades. Dept. Physiology, Northwestern Univ. Medical School, Chicago, Ill., and Dept. Anatomy, Emory Univ. School of Medicine, Ga.*
4. Biophysics of the cochlea. *B. E. Gernandt,* J. S. Riesco-McClure* and H. Davis. Central Institute for the Deaf, St. Louis.*
5. Functional disturbances and their compensation following circumscribed lesions of the vestibular nuclei. *S. R. Kemberling* and E. A. Spiegel. Depts. Exptl. Neurology and Pediatrics, Temple Univ. School of Medicine and Hospital, Philadelphia.*
6. Proprioception and convulsions. *J. Hyde, J. Gay and E. Gellhorn. Lab. of Neurophysiology, Dept. Physiology, Univ. of Minnesota, Minneapolis.*
7. Dark adaptation in lesions of the optic pathways. *Howard P. Krieger* and Morris B. Bender. Psychophysiological Lab., Dept. Neurology, New York Univ. College of Medicine, New York City.*
8. Light tonus of the human eye. *G. A. Brecher (introduced by C. J. Wiggers). Dept. Physiology, Univ. of Prague, Czechoslovakia, and Dept. Physiology, Western Reserve Univ. Medical School, Cleveland.*
9. Inhibition of activity of visual receptors by illuminating nearby retinal areas in the limulus eye. *H. K. Hartline. Dept. Biophysics, Johns Hopkins Univ., Baltimore, Md., and Johnson Research Foundation, Univ. of Pennsylvania, Philadelphia.*
10. Effect of bulbar facilitatory and inhibitory systems on spinal reflex activity. *L. M. N. Bach. Dept. Physiology, Tulane Univ. School of Medicine, New Orleans, La.*
11. Narcosis and refractory phase in certain cerebral mechanisms. *A. Forbes, A. F. Battista, P. O. Chatfield and J. P. Garcia. Harvard Medical School, Boston.*
12. Spinal cord pathways and connections for sensory fibers from the knee joint of the cat. *Ernest Gardner (introduced by M. Mason Guest). Dept. Anatomy, Wayne Univ. College of Medicine, Detroit.*
13. Bulbo-reticular stimulation and electrical activity of cerebral cortex. *G. Moruzzi* and H. W. Magoun. Dept. Anatomy, Northwestern Univ. Medical School, Chicago.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Kidney

COMMANDERY ASYLUM

1. Properties of antidiuretic substance in blood of normal and adrenalectomized rats. *J. H. Birnie,* W. J. Eversole,* W. R. Boss,* C. M. Osborn,* and Robert Gaunt. Dept. Zoology, Syracuse Univ., N. Y.*
2. Antidiuretic substance in the urine after fainting. *N. B. G. Taylor* and R. L. Noble. Collip Medical Research Laboratory, Univ. of Western Ontario, London, Canada.*
3. Renal excretion of phosphate in the dog; influence of K, action of parathyroid extract. *C. A. M. Hogben,* and J. L. Bollman. Mayo Foundation, Rochester, Minn.* (Pathol.).
4. Parathormone and tubular reabsorption of glucose and phosphate in man. *Walter H. Cargill and A. Calhoun Witham (introduced by James V. Warren). Depts. Physiology and Medicine, Emory Univ. School of Medicine, and Lawson V. A. Hospital, Atlanta.*
5. Urinary excretion of Ca during intravenous infusion. *Stanley M. Ball,* and A. V. Wolf. Dept. Physiology and Pharmacology, Albany Medical College, N. Y.*
6. Mechanisms of K excretion in the normal dog. *Robert W. Berliner, James G. Hilton and Thomas J. Kennedy, Jr. (introduced by James A. Shannon). Goldwater Memorial Hospital, and Dept. Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.*
7. Renal tubular K secretion in the normal dog. *Thomas J. Kennedy, Jr., James G. Hilton and Robert W. Berliner (introduced by James A. Shannon). Goldwater Memorial Hospital and Dept. Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.*
8. Occurrence of potassium U/P ratios lower than 1.0. *F. Mateer,* L. Greenman,* J. H. Peters,* R. C. Gow,* and T. S. Danowski. Dept. Research Medicine, Univ. of Pittsburgh School of Medicine, Penna.*
9. Renal secretion of K in the dog. *Gilbert H. Mudge,* James G. Foulks,* Adelbert Ames, III* and Alfred Gilman. Dept. Pharmacology, College of Physicians and Surgeons, Columbia Univ., New York City.*
10. Reabsorption of K by the kidney. *Robert Tarail and Donald W. Seldin (introduced by Donald H. Barron). Dept. Internal Medicine, Yale Univ. School of Medicine, New Haven.*
11. Site of action of mercurial diuretics. *John J. Duggan* and Robert F. Pitts. Dept. Physiology, Syracuse Univ. College of Medicine, N. Y.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Decompression; Acceleration; Blood Volume

CONSISTORY PARLOR

1. Repeated explosive decompressions. *David I. Mahoney (introduced by J. W. Wilson). Aero Medical Laboratory, Wright-Patterson Air Force Base, Dayton.*
2. Effects of explosive decompression on blood temperature in the right and left heart. *Edwin G. Vail* and Fred A. Hitchcock. Dept. Physiology, Ohio State Univ., Columbus.*
3. Factors correlated with resistance to decompression sickness. *True W. Robinson. Yellow Springs, Ohio.*
4. Muscular performance during positive radial acceleration. *Laurence E. Morehouse. Dept. Aviation Medicine, Univ. of Southern California School of Medicine, Los Angeles.*
5. Cerebral oxygen supply during positive acceleration. *J. P. Henry, O. Gauer,* E. E. Martin,* S. S. Kety* and K. Kramer.* Aero Medical Laboratory, Wright-Patterson Air Force Base, Dayton.*
6. Arterial oxygen saturation and intracardiac pressures during acceleration in relation to cardiac damage. *O. Gauer,* J. P. Henry, E. E. Martin* and P. J. Maher.* Aero Medical Laboratory, Wright-Patterson Air Force Base, Dayton.*
7. Static assessment of the relative efficiency of anti-G suits. *E. E. Martin,* G. Schroer* and J. P. Henry. Aero Medical Laboratory, Wright-Patterson Air Force Base, Dayton.*
8. Venous pressure as a factor limiting tolerance of acceleration in the prone position. *P. J. Maher, Jr.,* O. Gauer,* E. E. Martin* and J. P. Henry. Aero Medical Laboratory, Wright-Patterson Air Force Base, Dayton.*
9. Cause of injury in rapid deceleration. *Milton Joffe* and Fred A. Hitchcock. Dept. Physiology, Ohio State Univ., Columbus.*
10. Spectrophotometric determination of brilliant vital red and T-1824 in serum. *Philip Dow and Raymond W. Pickering.* Dept. Physiology, Univ. of Georgia School of Medicine, Augusta.*
11. Mixing time of brilliant vital red injected into dogs. *Raymond W. Pickering* and Philip*

Dow. Dept. Physiology, Univ. of Georgia School of Medicine, Augusta.

12. Reaction of T-1824 anion and T-1824 albumin with liver slices. *Thomas H. Allen and Peter D. Orahovats.* Dept. Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Gonads; Thyroid

ROOM 4

1. Effects of ovarian hormones on uterine pigmentation in vitamin E deficiency. *W. B. Atkinson,* H. Kaunitz and C. A. Slanetz.* Depts. Anatomy, Pathology and Animal Care, Columbia Univ. College of Physicians and Surgeons, New York City.* (Pathol.).
2. Tocopherol requirements during the rat's menopause. *Hans Kaunitz and C. A. Slanetz.* Depts. Pathology and Animal Care, Columbia Univ. College of Physicians and Surgeons, New York City.* (Pathol.).
3. Restoration of estrous cycle by alpha tocopherol in old rats. *S. Y. P'an, Hans Kaunitz and C. A. Slanetz.* Depts. Pharmacology, Pathology and Animal Care, Columbia Univ. College of Physicians and Surgeons, New York City.* (Pathol.).
4. Effect of low environmental temperature on thyroid function. *H. G. Schachner, Z. S. Gierlach and A. T. Krebs (introduced by H. Jensen). Medical Dept. Field Research Laboratory, Fort Knox.* (Biochem.).
5. Elemental iodine and iodocasein in hypothyroidism induced by methyl thiouracil. *Warren O. Nelson and Eileen B. Hines.* Dept. Anatomy, Univ. of Iowa, Iowa City.*
6. Quantitative measurement, by external counting, of the I^{131} content of the thyroid gland in man. *A. Stone Freedberg, Alvin Ureles and Marvin Van Dilla. Beth Israel Hospital and Harvard Medical School, Boston; and Massachusetts Inst. of Technology, Cambridge.*
7. Rise in serum precipitable or protein-bound iodine following massive doses of inorganic iodide. *T. S. Danowski and J. H. Greenman.* Dept. Research Medicine, Univ. of Pittsburgh School of Medicine, Penna.*
8. Metabolism of radioactive iodine in severe anoxic anoxia. *L. Van Middlesworth. Dept. Physiology, Univ. of Tennessee College of Medicine, Memphis.*
9. Radioactive diiodotyrosine in studies of thyroidal and tissue organic iodine. *Gopal Karandikar (introduced by William T. Salter). Dept. Pharmacology, Yale Univ. School of Medicine, New Haven.*
10. Distribution of microgram amounts of labeled thyroxine in the rat. *J. Gross and C. P. Leblond (introduced by S. M. Friedman). Dept. Anatomy, McGill University, Montreal, Canada.*
11. Action of thyroxine on protein metabolism. *J. Rupp,* K. E. Paschkis and A. Cantarow. Jefferson Medical College, Philadelphia.*
12. Evaluation of potential thyroxine inhibitors. *S. B. Barker, C. E. Kiely, Jr.,* S. Wawzonek* and S. C. Wang.* Depts. Physiology and Chemistry, Univ. of Iowa, Iowa City.*
13. Plasma and liver protein concentrations in adrenalectomized and in thiouracil-fed adrenalectomized rats. *J. H. Leathem. Bureau Biological Research and Dept. Zoology, Rutgers Univ., New Brunswick, N. J.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Heart; Circulation

ROOM 3

1. Spread of excitation and order of depolarization in the human heart. *L. H. Nahum and H. M. Chernoff.* Lab. of Physiology, Yale Univ. School of Medicine, New Haven.*
2. Analysis of electrocardiographic pattern produced by injection of epinephrine and norepinephrine in cats. *Eugene Lepeschkin and W. Raab (introduced by F. J. Sichel). Div. Exptl. Medicine, Univ. of Vermont College of Medicine, Burlington.*
3. Electrocardiographic findings in anthracosilicosis. *Leonard P. Lang* and Hurley L. Motley. Dept. Medicine, Jefferson Medical College and Cardio-Respiratory Laboratory, Barton Memorial Div. of Jefferson Hospital, Philadelphia.*
4. Behavior of actomyosin threads. *Samuel Mallov* and Jane Sands Robb. Dept. Pharmacology, Syracuse Univ. College of Medicine, N. Y.*
5. Electrograms of contracting actomyosin threads. *Jane Sands Robb and Samuel Mallov.* Dept. Pharmacology, Syracuse Univ. College of Medicine, N. Y.*
6. Residual electrocardiograms. *William G. Turman* and Jane Sands Robb. Dept. Pharmacology, Syracuse Univ. College of Medicine, N. Y.*
7. Order of recovery from excitation in dog, monkey, and human heart; nature of ST-segment and T-wave. *H. M. Chernoff* and L. H. Nahum. Lab. of Physiology, Yale Univ. School of Medicine, New Haven.*

8. Myocardial lactate and pyruvate metabolism in dogs under severe stress. *Walter T. Goodale and Donald B. Hackel (introduced by W. Fleischmann). Physiology Section, Medical Div., Army Chemical Center, Md.*
9. Myocardial metabolism in thiamine-deficient dogs studied by intravenous catheterization of the coronary sinus. *D. B. Hackel, W. T. Goodale and R. P. Johnson (introduced by W. Fleischmann). Physiology Section, Medical Div., Army Chemical Center, Md.*
10. Three-dimensional electrocardiography. *Raphael B. Levine* and Otto H. Schmitt. Depts. Zoology and Physics, Univ. of Minnesota, Minneapolis.*
11. Electrical analogs of the circulatory system. *Kenneth E. Jochim. Dept. Physiology, Univ. of Kansas, Lawrence.*
12. Effect of posterior pituitary extract on man. *F. Sunahara,* D. Duncanson* and O. G. Edholm. Dept. Physiology, Univ. of Western Ontario, London, Canada.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Neuro-Physiology

PARLOR F

1. Reflexology of the pudendal nerve field. *Charles H. Good (introduced by Berry Campbell). Dept. Anatomy, Univ. of Minnesota, Minneapolis.*
2. Excitability changes in dorsal roots produced by electrotonic effects from adjacent afferent activity. *Harry Grundfest and Jonathan Magnes.* Dept. Neurology, Columbia Univ. College of Physicians and Surgeons, New York City.*
3. Post-tetonic potentiation of presynaptic actions in the spinal cord. *David P. C. Lloyd. Laboratories of The Rockefeller Institute for Medical Research, New York City.*
4. Inhibition of the knee jerk from tendon spindles of crureus. *G. P. McCouch, I. D. Deering,* W. B. Stewart and W. W. Chambers.* Depts. Physiology and Anatomy, Univ. of Pennsylvania, Philadelphia.*
5. Postural reflexes in deafferented limbs of the cat. *R. D. Teasdall* and G. W. Stavraky. Dept. Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.*
6. Actions of dihydro-B-erythroidin and d-tubocurarine on neuromuscular and synaptic transmission in frog and crayfish. *Ernest B. Wright (introduced by W. O. Fenn). Dept. Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, N. Y.*
7. Suppressor action on primary sensory projection reactions. *Wade H. Marshall, National Institutes of Health, Bethesda, Md.*
8. Cortical response to light in the unanesthetized rabbit. *Reginald G. Bickford (introduced by Charles F. Code). Section on Physiology, Mayo Foundation and Mayo Clinic, Rochester, Minn.*
9. The motor cortex of the dog. *J. G. Chusid,* C. G. de Gutierrez-Mahoney and Franklin Robinson.* Neurological Div., St. Vincent's Hospital and Lab. of Physiology, Yale Univ. School of Medicine, New Haven.*
10. Responses elicited by combined stimulation of pairs of fixed electrodes in the unanesthetized monkey. *George Clark and James W. Ward.* Dept. Anatomy, Chicago Medical School, Ill.; Yerkes Laboratories of Primate Biology Inc., Orange Park, Fla.; and Dept. Anatomy, Vanderbilt Medical School, Nashville.*
11. Electrocorticograms of the cytoarchitectural areas of macaca mulatta. *John S. Garvin and L. V. Amador (introduced by Percival Bailey). Dept. Neurology and Neurological Surgery, Univ. of Illinois College of Medicine, and Illinois Neuropsychiatric Institute, Chicago.*
12. Effect of extirpating the prefrontal suppressor area 8s on correct olfactory conditioned differential responses in dogs. *William F. Allen. Dept. Anatomy, Univ. of Oregon Medical School, Portland.*

WEDNESDAY, APRIL 20, 7:00 P.M.

Special Meeting

MASONIC TEMPLE FOUNTAIN BALLROOM

WEDNESDAY, APRIL 20, 9:30 P.M.

Federation "Mixer"

HOTEL STATLER BALLROOM

THURSDAY, APRIL 21, 9:00 A.M.

Kidney

FOUNTAIN BALLROOM

1. Filtration rate and Na and H_2O excretion following mercurial diuretics. *Robert F.*

Pitts and John J. Duggan. Dept. Physiology, Syracuse Univ. College of Medicine.*

2. Effect of Pitressin and desoxycorticosterone in low dosage on excretion of H_2O and Na. *Otto W. Sartorius and Kathleen Roberts (introduced by R. F. Pitts). Dept. Physiology, Syracuse Univ. College of Medicine.*
3. Renal mechanism of albumin excretion in patients with nephrotic syndrome. *Henry D. Lauson, Francis P. Chinard* and Howard A. Eder.* Hospital of Rockefeller Institute for Medical Research, New York City.*
4. Renal clearances in normal dogs receiving DCA. *W. D. Collings, C. F. Downing* and R. E. Hodges.* Dept. Physiology, State Univ. of Iowa, Iowa City.*
5. Action of DOCA and adrenal cortical extract on body water and kidney function. *Mario Gaudino and Marvin F. Levitt (introduced by Homer W. Smith). Dept. Physiology, New York Univ. College of Medicine, New York City.*
6. Renal hemodynamics during short and long periods of salt-poor albumin administration. *Alexander J. Michie,* Nicholas S. Gimbel* and Cecilia Riegel. Harrison Dept. Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*
7. Effect of Bacitracin on renal function. *John H. Miller,* Roger K. McDonald* and Nathan W. Shock. National Heart Institute, Bethesda, Md., and Baltimore City Hospitals.*
8. Influence of graded arterial pressure decrement on renal clearance of PAH, creatinine, and Na. *Ewald E. Selkurt, Philip W. Hall* and Merrill P. Spencer.* Dept. Physiology, Western Reserve Univ. Medical School, Cleveland.*
9. Effect of increased renal venous pressure on renal function. *Rénė Wėgria, William D. Blake,* Richard P. Keating* and Henry P. Ward.* Dept. Medicine, Columbia Univ. College of Physicians and Surgeons, and Presbyterian Hospital, New York City.*
10. Effect of a psychiatric interview on renal plasma flow and finger skin temperature. *James H. Chalmers,* Robert W. Cranston,* Henry Longstreet Taylor and Ancel Keys. Laboratory Physiological Hygiene, Univ. of Minnesota, Minneapolis.*
11. Intestinal perfusion in a patient with chronic uremia. *N. S. R. Maluf. Brooke General Hospital, Fort Sam Houston, Texas.*
12. Effect of work on renal oxygen utilization. *John Kapp Clark and Harold G. Barker (introduced by J. R. Elkinton). Univ. of Pennsylvania School of Medicine, Philadelphia.*

THURSDAY, APRIL 21, 9:00 A.M.

Circulation

CONSISTORY PARLOR

1. Effect of l-arterenol on the peripheral circulation in man. *D. Duncanson,* T. Stewart* and O. G. Edholm. Univ. of Western Ontario Medical School, London, Ont., Canada.*
2. Hepatic venous circulation with special reference to the sphincter mechanism. *H. E. Essex and W. D. Thomas.* Mayo Foundation, Rochester, Minn.*
3. Constancy of vasomotor response to repeated intra-arterial injections of mecholyl and of epinephrine. *Lewis Franklin,* Howard Wayne,* J. Maxwell Little and Harold D. Green. Dept. Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem.*
4. Effect of sympathetic blocking procedures and of sympathectomy on peripheral blood flow. *S. W. Hoobler, J. W. Avera, W. J. Little, M. M. Peet and R. C. Bassett (introduced by A. H. Smith). Depts. Medicine and Surgery, Univ. of Michigan Hospital, Ann Arbor.*
5. Effect of increased intracranial pressure on the circulation in relation to pulmonary lesions. *Gilbert S. Campbell,* Francis J. Haddy* and M. B. Visscher. Dept. Physiology, Univ. of Minnesota, Minneapolis.*
6. Effect of hemorrhage on forearm blood flow and heart rate in man. *O. G. Edholm. Dept. Physiology, Univ. of Western Ontario, London, Canada.*
7. Determination of blood flow through the finger at high rates of flow. *Jere Mead and Robert C. Schoenfeld. Climatic Research Laboratory, Lawrence, Mass.*
8. Pressure and flow of blood distal to a point of reduced arterial bore. *Arnold H. Williams (introduced by Henry A. Schroeder). Dept. Internal Medicine and Oscar Johnson Institute, Washington Univ. School of Medicine, and Barnes Hospital, St. Louis.*
9. The law relating blood flow to perfusion pressure. *Harold Lamport. Lab. of Physiology, Yale Univ. School of Medicine, New Haven.*
10. 'Vertical tube' method for the study of pressure-flow relations in vascular beds. *J. Nichol,* F. Girling* and A. C. Burton. Dept. Biophysics, Univ. of Western Ontario, London, Canada.*
11. Development of turbulence in flowing blood. *N. A. Coulter* and John R. Pappenheimer. Dept. Physiology, Harvard Medical School, Boston.*

12. Oximetric measurement of circulation time and arterial saturation time during oxygen breathing in man. *Earl H. Wood, Bowen E. Taylor* and Julian Knutson.* Section on Physiology, Mayo Foundation, Univ. of Minnesota, Rochester.*

THURSDAY, APRIL 21, 9:00 A.M.

Neuro-Physiology

ROOM 4

2. Physiologic effects of bilateral simultaneous removal of Brodmann cytoarchitectural areas in the human. *Fred A. Mettler. Dept. Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.*
3. Pattern of localization in the motor cortex of the rat. *P. H. Settlage,* W. G. Bingham,* H. M. Suckle,* A. F. Borge* and C. N. Woolsey. Depts. Anatomy, Surgery and Physiology, Univ. of Wisconsin Medical School, Madison.*
4. Pattern of localization in the motor cortex of the marmoset. *C. N. Woolsey, P. H. Settlage,* H. M. Suckle* and W. G. Bingham.* Depts. Physiology, Anatomy and Surgery, Univ. of Wisconsin Medical School, Madison.*
5. Early components of contralaterally evoked cortical potentials in the intact unanesthetized rat. *Lowell A. Woodbury (introduced by J. E. P. Toman). Dept. Physiology, College of Medicine, Salt Lake City.*
6. Development of seizure pattern by repetitive minimal cortical stimulation. *J. A. Whieldon* and A. van Harreveld. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena.*
7. Differences in cortically and subcortically induced convulsions. *Ernst Gellhorn. Lab. of Neurophysiology, Dept. Physiology, Univ. of Minnesota, Minneapolis.*
8. Fractional conditioning of behavior based on electrically induced convulsions. *Stephen Fleck* and W. Horsley Gantt. Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Univ., Baltimore.*
9. Anoxia and conditional reflexes in dogs. *W. Horsley Gantt, George W. Thorn and C. Dorrance. Pavlovian Laboratory, Phipps Psychiatric Clinic, and Dept. of Medicine, Johns Hopkins Univ., Baltimore.*
10. Centers governing temperature selection and frequency response to temperature in the brain of trout. *Charlotte Sullivan* and Kenneth C. Fisher. Univ. of Toronto, Canada.*
11. Developmental relationships between electrical activity and histogenesis of the cerebellum. *John Jacobs and Ray S. Snider (introduced by H. W. Magoun). Depts. Pathology and Anatomy, Northwestern Univ. Medical School, Chicago.*
12. Activity of single units in the cerebellar cortex. *G. Moruzzi,* John M. Brookhart and R. S. Snider. Depts. Anatomy and Physiology, Northwestern Univ. Medical School, Chicago.*

THURSDAY, APRIL 21, 9:00 A.M.

Blood Volume; Heat Regulation

ROOM 3

1. Concurrent estimates of blood volume in rabbits by bleeding and dye methods. *Paul L. McLain, C. H. William Ruhe* and T. K. T. Kruse. Dept. Physiology and Pharmacology, School of Medicine, Univ. of Pittsburgh, Penna.*
2. Influence of repeated artificial pneumothorax on hematocrit values and plasma volume. *Wilson C. Grant (introduced by Walter S. Root). Dept. Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*
3. Effect of spinal anesthesia on circulating blood volume. *S. J. Sarnoff, E. S. Buckley, Jr., J. L. Whittenberger and J. G. Gibson, II (introduced by W. H. Forbes). Dept. Physiology, Harvard School of Public Health, and Dept. Medicine, Harvard Medical School, Boston.*
4. Analysis of out-of-doors thermal stress due to radiation with the pan-radiometer. *Charles H. Richards* and James D. Hardy. New York Hospital, and Dept. Physiology, Cornell Univ. Medical College, New York City.*
5. Thermistors as applied to temperature measurements in animals. *J. F. Herrick and E. Glarborg. The Mayo Foundation, Rochester, Minn.*
6. Regional distribution of cutaneous heat losses. *Alrick B. Hertzman and Walter C. Randall. Dept. Physiology, St. Louis Univ. School of Medicine, St. Louis.*
7. Relations between blood flow, skin temperature, evaporation rates and sweating in various skin regions. *Walter C. Randall and Alrick B. Hertzman. Dept. Physiology, St. Louis Univ. School of Medicine, St. Louis.*
8. Responses to vagal stimulation during hyperthermia. *H. E. Ederstrom (introduced by A.*

B. Hertzman). *Dept. Physiology, St. Louis Univ. School of Medicine, St. Louis.*

9. Responses of man to internal thermal stimulation. *Austin Henschel, Henry Longstreet Taylor and Ancel Keys. Laboratory of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*
10. Comparison of intracardiac and intravascular temperatures with rectal temperature. *Ludwig W. Eichna, Adolph R. Berger and Bertha Rader (introduced by David P. Earle, Jr.). Dept. Medicine, New York Univ. College of Medicine, New York City.*
11. Blood temperature in the heart and blood vessels of the dog. *Steven M. Horvath, E. L. Foltz,* A. Rubin* and B. K. Hutt.* Dept. Physical Medicine and Pharmacology, Univ. of Pennsylvania, Philadelphia.*
12. Heating of tissues by near and far infrared radiation. *Jerome W. Gersten,* Khalil G. Wakim, Richard W. Stow,* and Adrien N. Porter.* Sections on Physical Medicine and Physiology, Mayo Foundation and Mayo Clinic, Rochester, Minn.*

THURSDAY, APRIL 21, 9:00 A.M.

Protein Metabolism

PARLOR F

1. Further lowering of glucose tolerance following an induced hypothyroid state in Yale rats. *Thomas F. Frawley and Charles W. Bishop (introduced by George F. Koepf). Dept. Medicine, Univ. of Buffalo School of Medicine, and Medical Research Labs., Buffalo General Hospital, N. Y.*
2. Resistance induced by anti-thyroid compounds and by goitrogenic diets against experimental pulmonary edema in rats. *K. K. Carroll* and R. L. Noble. Dept. Medical Research, Collip Medical Research Lab., Univ. of Western Ontario, London, Canada.*
3. Influence of biliary obstruction upon liver protein regeneration. *Harry M. Vars, Colin C. Ferguson* and Charles S. Rogers.* Harrison Dept. Surgical Research, Univ. of Pennsylvania Schools of Medicine, Philadelphia* (Biochem.).
4. Effect of dietary protein on cholic acid synthesis in dogs. *J. H. Annegers and F. Friend (introduced by John S. Gray). Dept. Physiology, Northwestern Univ. Medical School, Chicago, Ill.*
5. Role of insulin in the metabolism of amino acids. *William D. Lotspeich. Syracuse Univ. College of Medicine, N. Y.*
6. Metabolism of oxypurines in man. *William Q. Wolfson,* Clarence Cohn and Kinu Kadota.* Dept. Biochemistry, Medical Research Institute, Michael Reese Hospital, Chicago.*
7. Concurrent feeding of amino acids. *Richard Henderson* and Robert S. Harris. Nutritional Biochemistry Labs., Massachusetts Institute of Technology, Cambridge.*
8. A procedure for the *in vitro* study of hormonal influences in nitrogen metabolism. *Daniel L. Kline (introduced by Jane A. Russell). Dept. Physiological Chemistry, Yale Univ. School of Medicine, New Haven.*
9. Castration and the mobilization of nitrogen during fasting. *Clara M. Szego and Abraham White. Dept. Physiological Chemistry, Univ. of California School of Medicine, Los Angeles.*
10. Incorporation of glycine in tissues of acutely starved rats. *T. R. Noonan, K. I. Altman, G. W. Casarett and K. Salomon (introduced by William F. Bale). Dept. Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, N. Y.*
12. Inhibition of growth of mice on a low protein, choline-deficient diet by coramine. *J. Walter Wilson and Elizabeth H. Leduc (introduced by Paul F. Fenton). Brown Univ., Providence.*

THURSDAY, APRIL 21, 1:30 P.M.

Kidney

FOUNTAIN BALLROOM

1. Passage of hemoglobin through glomerular membranes and its excretion by the amphibian kidney. *Phyllis A. Bott. Dept. Physiological Chemistry, Woman's Medical College of Pennsylvania, Philadelphia* (Biochem.).
2. Can transfer rate be calculated from urine isotope ratio? *Herbert D. Friendlander* and Walter S. Wilde. Physiology Dept., Tulane Univ. School of Medicine, New Orleans.*
3. Age and osmotic work in urea excretion. *D. F. Davies (introduced by H. A. Schroeder). Section on Cardiovascular Disease and Gerontology, National Institutes of Health, Bethesda, Md., and Baltimore City Hospitals.*

4. A method for determination of volume of distribution of metabolized substances. *Irving L. Schwartz, Murray Rabinowitz and David Schachter (introduced by Homer W. Smith). Dept. Physiology, New York Univ. College of Medicine, New York City.*
5. Body water compartments in man. *Eugene Y. Berger,* Marcelle F. Dunning,* Bernard B. Brodie* and J. Murray Steele. Depts. Medicine and Biochemistry, New York Univ. College of Medicine, and Research Service, Third New York Univ. Medical Division, Goldwater Memorial Hospital, New York City.*
6. Transcapillary exchange of inulin in man. *Irving L. Schwartz, David Schachter and Norbert Freinkel (introduced by Homer W. Smith). Dept. Physiology, New York Univ. College of Medicine, New York City.*
7. Effect of low protein diet and plasmapheresis on fluid compartments and glomerular filtration rate. *Marjorie B. Zucker, Louis Cizek* and Douglas Tompkins.* Dept. Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*
8. Mixing rate within the mobile phosphate pool of cows. *Max Kleiber, A. H. Smith,* N. P. Ralston* and D. E. Jasper.* College of Agriculture, Univ. of California, Davis.*
9. Lymph flow during edema formation. *George Fahr and Herman Koschnitzke. Minneapolis General Hospital, Minn.*
10. Observations on pulmonary lymph flow and edema. *Robert Paine,* Frank A. Howard,* Harvey R. Butcher* and John R. Smith. Cardiovascular Division, Dept. Medicine, Washington Univ. School of Medicine, St. Louis.*
11. Effect of hydration on survival of fasting rats. *Dorothy Nelson (introduced by A. C. Ivy). Dept. Clinical Sciences, Univ. Illinois College of Medicine, Chicago.*
12. Reduction of plasma K-concentration by vivodialysis and its restoration in non-visceral regions. *Roger M. Reinecke and Francis L. Stutzman.* Dept. Physiology, Univ. of Minnesota, Minneapolis.*
13. Measurement of intracellular cation in the dog. *Marvin F. Levitt and Mario Gaudino (introduced by Homer W. Smith). Dept. Physiology, New York Univ. College of Medicine, New York City.*

THURSDAY, APRIL 21, 1:30 P.M.

Circulation

CONSISTORY PARLOR

1. Pulmonary blood flow and alveolar ventilation-perfusion relationship following resection of one lung in man. *A. Cournand, R. L. Riley and A. Himmelstein.* Depts. Medicine and Surgery, Columbia Univ. College of Physicians and Surgeons, New York City.*
2. Transmission spectra of the human ear (pinna and lobe) at arterial occlusive pressures. *J. F. Neville, Jr., J. O. Elam, K. Sugioka, and A. Roos (introduced by H. L. White). Lab. of Applied Thoracic Physiology and Dept. Physiology, Washington Univ. School of Medicine, St. Louis.*
3. Comparison of transmission spectra of blood in the human ear flushed by histamine with those obtained after heat flushing. *William Sleator, Jr., James O. Elam, D. J. Kilian, W. N. Elam, Jr. (introduced by H. L. White). Dept. Physiology and Lab. of Applied Thoracic Physiology, Washington Univ. School of Medicine, St. Louis.*
4. Photo-electric measurements of arterial oxygen saturation dynamics in man for study of cardio-pulmonary function. *J. O. Elam,* J. L. Ehrenhaft,* W. N. Elam, Jr.,* and H. L. White. Lab. of Applied Thoracic Physiology and Dept. Physiology, Washington Univ. School of Medicine, St. Louis.*
6. Reflex respiratory and circulatory action of veratridine on cardiac, pulmonary and carotid receptors. *Domingo M. Aviado, Jr. and Robert G. Pontius (introduced by Carl F. Schmidt). Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*
7. Alteration of the pH of the blood as a means of improving experimental cardiac anoxia. *Kurt Lange, Victor Tohertkoff, Frank Craig and David Weiner (introduced by M. G. Mulinos). Dept. Medicine, New York Medical College, New York City* (Pharmacol.).
8. Position of the oxygen dissociation curve in cyanotic congenital heart disease. *Minerva Morse, Donald E. Cassels* and Melba Holder.* Dept. Pediatrics, Univ. Chicago* (Biochem.).
9. Action of caffeine and aminophylline as respiratory stimulants in man. *Glenn H. Richmond (introduced by C. L. Gemmill). Dept. Pharmacology, Univ. of Virginia Medical School, Charlottesville* (Pharmacol.).
10. Hemodynamic changes in response to hemorrhage with and without dibenamine. *John W. Remington. Dept. Physiology, Univ. of Georgia School of Medicine, Augusta.*
11. Effects of shock (low arterial blood pressure) on the pulmonary vessel pressures. *Francis J. Haddy,* Gilbert S. Campbell* and M. B. Visscher. Dept. Physiology, Univ. of Minnesota, Minneapolis.*
12. Predisposition to vasodepressor properties

of morphine induced by hemorrhagic hypotension. *Richard E. Lee* and Benjamin W. Zweifach. Dept. Pharmacology, Syracuse Medical College, N. Y., and Dept. Medicine, New York Hospital, New York City.*

13. Effects of experimental anaemia on the circulation in dogs. *J. D. Hatcher,* F. Sunahara,* J. C. van Noordwijk* and O. G. Edholm. Dept. Physiology, Univ. Western Ontario, London, Canada.*

THURSDAY, APRIL 21, 1:30 P.M.

Neuro-Physiology

ROOM 4

1. Action potentials of the cerebellar cortex elicited by stimulation of olivo-cerebellar fibers. *Gordon M. Schoepfle. Dept. Physiology, Washington Univ. School of Medicine, St. Louis.*
2. Localization of the central nervous mechanism of emesis. *H. L. Borison* and S. C. Wang. Dept. Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*
3. A skin resistance recorder or sudomotometer. *William A. Geohegan (introduced by Joseph C. Hinsey). Dept. Anatomy, Cornell Univ. Medical College, New York City.*
4. Acute renal cortical ischemia produced by stimulation of the pressor area of the cerebral cortex. *E. C. Hoff, J. F. Kell, Jr.,* Nelson Hastings,* E. H. Gray* and D. M. Sholes.* Neurological Science Lab., Medical College of Virginia, Richmond.*
5. Respiratory and vascular responses in monkeys from temporal pole, insula, orbital surface and cingulate. *B. R. Kaada (Univ. of Oslo, Norway), K. H. Pribram and J. A. Epstein (introduced by John F. Fulton). Lab. of Physiology, Yale Univ. School of Medicine, New Haven.*
6. Alterations in segmental sympathetic (sweat gland) activity through myofascial and postural disturbances. *Irvin M. Korr. Kirksville College of Osteopathy and Surgery, Kirksville, Mo.*
7. Effects of bacterial polysaccharides on cardiovascular and neuromuscular systems. *Chester E. Leese and K. Virginia Greene.* Dept. Physiology, George Washington Univ. School of Medicine, Washington, D. C.*
8. Separate inhibition of resting and active oxygen consumption of functioning nerves. *R. W. Doty* and R. W. Gerard. Dept. Physiology, Univ. of Chicago.*
9. Utilization of fructose by the perfused brain of the living cat. *A. Geiger, J. Magnes, R. M. Taylor, and H. Waelsch (introduced by H. Grundfest). Depts. Biochemistry and Psychiatry, New York State Psychiatric Institute, New York City.*
10. Effect of blood composition on the metabolic activity of the perfused brain in the living cat. *J. Magnes, R. M. Taylor and A. Geiger (introduced by H. Grundfest). Dept. Psychiatry, Columbia Univ. College of Physicians and Surgeons, New York City.*
11. Phosphate turnover in the mammalian nervous system. *A. J. Samuels,* L. L. Boyarsky,* B. Libet, R. W. Gerard and M. Brust.* Dept. Physiology, Univ. of Chicago.*
12. Metabolism of the rat spinal cord functioning in isolation. *R. D. Tschirgi,* R. W. Gerard, H. Jenerick,* L. L. Boyarsky* and J. Z. Hearon.* Dept. Physiology, Univ. of Chicago.*
13. Effect of dinitrophenol on respiration of excised brains of rats at various stages of development. *David B. Tyler. Dept. Embryology, Carnegie Institution of Washington, Baltimore.*

THURSDAY, APRIL 21, 1:30 P.M.

Coagulation

ROOM 3

1. Species reactivity of staphylocoagulase and staphylokinase. *Earl B. Gerheim* and John H. Ferguson. Dept. Physiology, Univ. North Carolina, Chapel Hill.*
2. Action of heparin on protoplasmic gelation. *L. V. Heilbrunn. Dept. Zoology, Univ. Pennsylvania, Philadelphia.*
3. Inhibition of a proteolytic enzyme system of blood. *Jessica H. Lewis* and John H. Ferguson. Dept. Physiology, Univ. North Carolina, Chapel Hill.*
4. Clotting mechanism in relation to protamine sulfate and heparin. *Peter V. Moulder, Burton J. Grossman and Margaret H. Sanderson (introduced by J. Garrott Allen). Dept. Surgery, Univ. of Chicago.*
5. Method for the determination of heparin in blood. *F. C. Monkhouse,* Mary Stewart* and L. B. Jaques. Dept. Physiology, Univ. Saskatchewan, Saskatoon, Canada.*
6. Amount of protamine sulfate required to reestablish *in vitro* clotting of heparinized blood. *Charles L. McKeen, Richard M. Elghammer and Peter V. Moulder (introduced by J. Garrott Allen). Dept. Surgery, Univ. of Chicago.*

7. Action of phenylindanedione on prothrombin time. *L. B. Jaques, E. Taylor* and E. Lepp.* Dept. Physiology, Univ. of Saskatchewan, Saskatoon, Canada.*
8. Action of antitrypsin and antifibrinolysin on the first phase of the blood clotting reaction. *M. Mason Guest and Thomas E. Nelson.* Dept. Physiology and Pharmacology, Wayne Univ. College of Medicine, Detroit.*
9. Site of action of soy bean trypsin inhibitor on the coagulation system of blood. *Mary Beth Glendening* and Ernest W. Page. Dept. Obstetrics and Gynecology, Univ. of California Medical School, San Francisco.*
10. An anti-coagulant present in testicular extracts. *S. Fisch and E. J. Towbin (introduced by E. F. Adolph). Dept. Physiology, Univ. of Rochester, N. Y.*
11. Changes in clotting mechanism produced by protamine sulfate and heparin. *J. Garrott Allen and Willadene Egner.* Dept. Surgery, Univ. of Chicago, Ill.*
12. Inactivation of thromboplastin by serum. *F. D. Mann and Margaret Hurn (introduced by T. B. Magath). Division Clinical Laboratories, Mayo Clinic, Rochester, Minn.*

THURSDAY, APRIL 21, 1:30 P.M.

Heat Regulation

PARLOR F

1. Measurement of cold and warmth thresholds of subjects exposed to environments from 18°C. to 38°C. *Franklin G. Ebaugh, Jr. and Rudolf Thauer (introduced by Eugene F. Du Bois). New York Hospital and Depts. Medicine and Physiology, Cornell Univ. Medical College, New York City.*
2. Effect of ethyl carbamate on temperature regulation. *Ronald Grant and Marilyn E. Robbins (introduced by Victor E. Hall). Dept. Physiology, Stanford Univ., Calif.*
3. Measurement of sympathetic denervation in man by sweating patterns after sympathectomy. *Grace M. Roth and Winchell McK. Craig.* Sections on Clinical Physiology and Neurologic Surgery, Mayo Clinic, Rochester, Minn.*
4. Dynamics of evaporative heat loss for a rapidly cooling human body. *Tohru Inouye,* Nathaniel Glickman and Robert W. Keeton. Dept. Medicine, Univ. of Illinois, Chicago.*
5. Digital skin temperature and blood flow relationship following change in environmental temperature. *Harwood S. Belding, Jere Mead* and Mortimer E. Bader.* QMC Climatic Research Laboratory, Lawrence, Mass.*
6. Mechanism of cardiac injury in experimental hypothermia. *David Weiner, Kurt Lange and Michael M. A. Gold (introduced by M. G. Mulinos). Dept. Medicine, New York Medical College, New York City* (Pharmacol.).
7. Metabolism pf pigeons in extreme cold. *E. Streicher,* D. B. Hackel,* W. Fleischmann and G. D. Bulger.* Physiology Section, Medical Division, Army Chemical Center, Md.*
8. Effect of altered metabolism on extreme hypothermia in dogs and albino rats. *Richard C. Wolff* and K. E. Penrod. Dept. Physiology, Boston Univ. School of Medicine.*
9. Pulse rate and blood pressure of dogs in immersion hypothermia. *A. H. Hegnauer and H. O. Haterius. Dept. Physiology, Boston Univ. School of Medicine.*
10. Effect of immersion hypothermia on cardiac output. *Felix R. Rosenhain* and K. E. Penrod. Dept. Physiology, Boston Univ. School of Medicine.*
11. Blood gas content of dogs during immersion hypothermia. *K. E. Penrod and Felix R. Rosenhain.* Dept. Physiology, Boston Univ. School of Medicine.*
12. Effect of hypothermia on cardiodynamics. *William J. Shriber (introduced by A. H. Hegnauer). Dept. Physiology, Boston Univ. School of Medicine.*
13. Effect of hypothermia on bromsulphalein removal. *Radford Brokaw (introduced by K. E. Penrod). Dept. Physiology, Boston Univ. School of Medicine.*

THURSDAY, APRIL 21, 1:30 P.M.

Gastro-Enterology

MURAL ROOM

1. Gastric distention as a factor in satiation of thirst. *E. J. Towbin (introduced by E. F. Adolph). Physiology Dept., Univ. of Rochester, N. Y.*
2. Regulation of food intake in normal and esophagostomized dogs. *Henry D. Janowitz* and M. I. Grossman. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
3. Action of enzyme inhibitors on activity of isolated intestine. *A. Farah, R. Angel* and T. C. West.* Dept. Pharmacology, Univ. of Washington School of Medicine, Seattle* (Pharmacol.).
4. Pressure and activity recordings in pylorus and rectum of man. *F. R. Steggerda and W. C. Clark.* Dept. Physiology, Univ. of Illinois, Urbana.*

5. 'Dumping syndrome' in the dog following anastomotic operations and vagotomy. *N. C. Jefferson,* C. W. Phillips,* R. Levine and H. Necheles. Dept. Gastro-Intestinal Research, Medical Research Institute, Michael Reese Hospital, Chicago.*
6. Gastrointestinal motor effects of intestinal extracts. *M. H. F. Friedman. Dept. Physiology, Jefferson Medical College, Philadelphia.*
7. Intravenous injection of hypertonic saline salivary secretion in dogs. *L. J. Cizek* and M. I. Gregersen. Dept. Physiology, Columbia Univ. College of Physicians and Surgeons, New York City.*
8. Influence of hesperidin methyl chalcone on blood flow and secretion of the salivary gland. *David F. Bohr and W. John Kenfield (introduced by Robert Gesell). Dept. Physiology, Univ. of Michigan, Ann Arbor.*
9. Effect of sham-feeding on gastric motor activity of the dog. *Stanley H. Lorber,* S. A. Komarov and Harry Shay. Samuel S. Fels Research Institute, Temple Univ. School of Medicine, Philadelphia.*
10. K deficiency and gastrointestinal motility. *Helen A. Winter,* Hebbel E. Hoff, and Leslie Dso.* Dept. Physiology, McGill Univ., Montreal, Canada.*
11. Action of epinephrine on the pyloric sphincter as recorded by the pyloric inductograph. *Hortense Louckes,* Daniel A. Brody* and J. P. Quigley. Division of Physiology, Univ. of Tennessee, Memphis.*
12. Effect of tetraethylammonium on gastric motility of the dog. *Ardelle Lane,* C. R. Robertson* and M. I. Grossman. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
13. Effect of prostigmine and urecholine on human gastric motility. *N. C. Hightower, Jr.,* C. F. Code, F. T. Maher,* and C. G. Morlock.* Mayo Foundation and Mayo Clinic, Rochester, Minn.*

THURSDAY, APRIL 21, 4:30 P.M.

Physiology Business Meeting

FOUNTAIN BALLROOM

FRIDAY, APRIL 22, 9:00 A.M.

Blood Pressure

FOUNTAIN BALLROOM

1. An electronic, multi-range, multi-channel, direct-writing pressure recorder. *Maurice Rappaport and Stanley J. Sarnoff (introduced by William H. Forbes). Dept. Physiology, Harvard School of Public Health, Boston.*
2. A method for correlating blood pressure with changes in the peripheral vascular bed of unanesthetized mice. *Glenn H. Algire. National Cancer Institute, National Institutes of Health, Bethesda, Md.* (Pathol.).
3. Cardio-vascular effects of nembutal, dibenamine and yohimbine on trained dogs. *George Constant,* Aristophanes Andronis* and Eric Ogden. Dept. Physiology, Univ. of Texas Medical Branch, Galveston.*
4. Rapid spontaneous variations in blood pressure. *Henry A. Schroeder. Dept. Internal Medicine, Washington Univ. School of Medicine, and Barnes Hospital, St. Louis.*
6. Relationships between the blood pressure increases in exercise, CO_2 inhalation and the cold pressor test. *Henry Longstreet Taylor, Austin Henschel, Josef Brozek and Ancel Keys. Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*
7. Blood pressure changes produced by local thermal stimulation of the brain. *S. Rodbard, L. Taylor* and D. Ferguson.* Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago.*
8. Potent pressor action of an extract of a phaeochromocytoma after adrenolytic doses of dibenamine. *D. M. Pitcairn* and W. B. Youmans. Dept. Physiology, Univ. of Oregon Medical School, Portland.*
9. Effect of a psychiatric interview on the blood pressure response to cold stimuli. *Robert W. Cranston,* James H. Chalmers,* Henry Longstreet Taylor, Austin Henschel and Ancel Keys. Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*
10. Mechanisms of the vascular action of tetraethylammonium chloride. *Irvine H. Page, Ralph Prince* and John J. Reinhard, Jr.* Research Division and Bunts Institute of the Cleveland Clinic Foundation, Cleveland.*
11. Hypotensive effects of intravenous injections of hypertonic solutions. *Ingrith J. Deyrup* and William W. Walcott. Dept. Zoology, Barnard College, and Dept. Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.*
12. Influence of low protein diets on renal VEM formation. *Mary Ann Payne* and Ephraim Shorr. Dept. Medicine, Cornell Univ. Medical College, and the New York Hospital, New York City.*

FRIDAY, APRIL 22, 9:00 A.M.

Heat Regulation; CHO Metabolism

CONSISTORY PARLOR

1. Heat loss and heat production of cats at different environmental temperatures. *Lawrence R. Prouty. Dept. Physiology, Cornell Univ. Medical College, New York City.*
2. Ascorbic acid and acclimatization of animals to cold environment. *M. Thérien* and L. P. Dugal. Dépt. d'Acclimatation, Institut de Biologie Humaine, Univ. Laval, Québec, Canada.*
3. Effect of local cooling and heating of the finger and wrist during exposure to high ambient temperature. *Mortimer E. Bader and Jere Mead (introduced by H. S. Belding). Quartermaster Corps, Climatic Research Laboratory, Lawrence, Mass.*
4. Efficiency of the excretory system in desert mammals. *Knut Schmidt-Nielsen (introduced by Laurence Irving). Dept. Physiology, Stanford Univ., Calif. and Arctic Research Laboratory.*
5. Reduced evaporative water loss from the lungs of certain desert mammals. *Bodil Schmidt-Nielsen (introduced by Laurence Irving). Dept. Physiology, Stanford Univ., Calif., and Arctic Research Laboratory.*
6. Pulsatile evaporative rates from small skin areas as measured by an infra red gas analyzer. *R. E. Albert and E. D. Palmes (introduced by A. D. Keller). Medical Dept., Field Research Laboratory, Fort Knox.*
7. Carbohydrate metabolism in the decerebrated rat. *Evelyn Anderson and Webb Haymaker.* National Institute of Health, Bethesda, Md., and Army Institute of Pathology, Washington, D. C.*
8. Ketosis in diabetics in response to the stress of interpersonal adjustment. *Lawrence E. Hinkle, Jr. and George T. Conger (introduced by Stewart Wolf). Depts. Medicine and Psychiatry, New York Hospital and Cornell Univ. Medical College, New York City.*
9. Effect of carbohydrate on starvation metabolism in the presence of a high caloric deficit. *G. W. Mellinger (introduced by J. W. Heim). Aero Medical Laboratory, Wright-Patterson Air Force Base, Dayton.*
10. Metabolism of lactate by the isolated dog gastrocnemius. *Nathan Lifson, Akira Omachi* and Shirley L. Michel.* Dept. Physiology, Univ. of Minnesota, Minneapolis.*
11. Reduced synthesis of phosphocreatine in tissue homogenates from alloxan diabetic rats. *E. S. Goranson (introduced by R. E. Haist). Dept. Physiology, Univ. of Toronto, Canada.*
12. Effects of growth hormone on the blood sugar and insulin sensitivity of the rat. *Anne E. Milman* and Jane A. Russell. Dept. Physiological Chemistry, Yale Univ., New Haven.*

FRIDAY, APRIL 22, 9:00 A.M.

Gastro-Enterology

ROOM 4

1. Effect of vagotomy on gastric secretion and emptying time in the dog. *F. Antia* and A. C. Ivy. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
2. Effect of urine extracts from hypophysectomized dogs on gastric sections. *T. L. Patterson, J. Kaulbersz, D. J. Sandweiss and H. C. Saltzstein.* Depts. Physiology and Surgery, Wayne Univ. College of Medicine, and Harper Hospital, Detroit.*
3. Interrelation of effects of intraarterial injections of histamine and acetylcholine on gastric section. *George W. Stavraky. Dept. Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Canada.*
4. Effect of some xanthine derivatives on gastric secretion. *C. R. Robertson* and A. C. Ivy. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
5. Bile action on volume and content of gastric juice in triple histamine experiments. *Jerzy Kaulbersz and Ryszard Bilski. Dept. Physiology, Jagellonian Univ., Cracow, Poland.*
6. Acid secretion by gastric mucosa of the bull frog in vitro. *Leslie E. Edwards and Carolyn Trowbridge Edwards (introduced by Robert W. Ramsey). Medical College of Virginia, Richmond.*
7. Effects of certain physical factors on in vitro gastric secretion of the dog. *Carolyn Trowbridge Edwards and Leslie E. Edwards (introduced by Robert W. Ramsey). Dept. Physiology and Pharmacology, Medical College of Virginia, Richmond.*
8. Pepsin content of histamine-stimulated gastric juice. *Gladys R. Bucher. Depts. Biological Sciences and Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
9. Mucoproteins of gastric juice and mucus and mechanism of their secretion. *Allan Grossbert,* S. A. Komarov and Harry Shay. Samuel S. Fels Research Institute, Temple Univ. School of Medicine, Philadelphia.*
10. Fractionation of enterogastrone concentrates

with N-butyl alcohol. *Leon L. Gershbein,* Hugh M. Millen* and A. C. Ivy. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*

11. Analysis of factors affecting assay of gastric secretory depressants. *D. Blickenstaff* and M. I. Grossman. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
12. Occurrence of gastric secretory-inhibitor activity in fresh gastric and salivary mucin. *Charles F. Code, Henry V. Ratke,* George R. Livermore, Jr.* and Walter Lundberg.* Mayo Foundation, Rochester, Minn., and Hormel Institute, Univ. of Minnesota, Minneapolis.*

FRIDAY, APRIL 22, 9:00 A.M.

Coagulation; Blood Elements

ROOM 3

1. Effect of adrenoxyl on blood loss from surgical wounds. *R. F. Hagerty and J. J. Zavertnik (introduced by K. S. Grimson). Dept. Surgery, Duke Univ., Durham.*
2. Serum prothrombic activity and prothrombin conversion accelerator in hemophilia and thrombocytopenia. *Benjamin Alexander and Greta Landwehr (introduced by Herrman L. Blumgart). Beth Israel Hospital and Harvard Medical School, Boston.*
3. Transition of fibrinogen to fibrin. *Koloman Laki (introduced by William J. Bowen). Institute of Biochemistry, Budapest, Hungary, and National Institutes of Health, Bethesda, Md.*
4. A new method of fibrin determination and applications to study of thrombin-fibrinogen reaction. *Paul W. Boyles* and John H. Ferguson. Dept. Physiology, Univ. of North Carolina, Chapel Hill.*
5. Hemorrhagic diathesis in leukemia. *Maryloo Spooner (introduced by W. J. Meek). Dept. Physiology, Univ. of Wisconsin Medical School, Madison.*
6. Experimental production of hemorrhages in dogs with aminopterin, and their control with toluidine blue. *Burton J. Grossman, Margaret H. Sanderson and Willadene Egner (introduced by J. Garrott Allen). Dept. Surgery, Univ. of Chicago.*
7. Coagulation decelerating action of hemophilic plasma on normal plasma. *L. M. Tocantins and R. T. Carroll.* Division of Hematology, Dept. Medicine, Jefferson Medical College, Philadelphia.*
8. Blood coagulation in relation to menstruation and menorrhagia. *Richard M. Elghammer,* Burton J. Grossman,* Peter V. Moulder,* Arthur K. Koff* and J. Garrott Allen. Dept. Surgery, Univ. of Chicago.*
9. Use of fraction I for separation of erythrocytes and leukocytes from whole blood. *Edward S. Buckley, Jr., Marvin J. Powell and John G. Gibson, 2nd. Peter Bent Brigham Hospital and Harvard Medical School, Boston.*
10. Blood production and iron reserves in man. *Stuart Finch, Donald Haskins and Clement A. Finch (introduced by George W. Thorn). Harvard Medical School and Peter Bent Brigham Hospital, Boston.*
11. Electrolyte, nitrogen and protein changes after massive removal of red cells and plasma protein. *Charles L. Fox, Jr. and Betty B. Freeman (introduced by Alfred Gilman). Dept. Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.*
12. Hemoglobin iron as a stimulus for production of ferretin. *John K. Hampton, Jr. (introduced by H. S. Mayerson). Dept. Physiology, Tulane Univ. School of Medicine, New Orleans.*

FRIDAY, APRIL 22, 9:00 A.M.

Muscle

PARLOR F

1. Effect of abdominal ice packs on recovery from fatigue. *W. W. Tuttle, W. P. Happ* and M. Wilson.* Dept. Physiology, State Univ. of Iowa, Iowa City.*
2. Action potential of heart muscle. *Howard J. Curtis. Dept. Physiology, Vanderbilt Univ. Medical School, Nashville.*
3. Ratio between tension and total electrical activity in human skeletal muscle. *R. Snyder,* C. F. S. Fisher* and A. C. Burton. Dept. Biophysics, Univ. of Western Ontario, London, Ont., Canada.*
4. Action potentials in single muscle fibers. *W. L. Nastuk and A. L. Hodgkin (introduced by M. I. Gregersen). Dept. Physiology, College of Physicians and Surgeons, Columbia Univ., New York City, and the Physiological Lab., Cambridge Univ., England.*
5. Single muscle fiber membrane potential. *G. Ling and R. W. Gerard. Dept. Physiology, Univ. of Chicago.*
6. Cooling as a stimulus to certain smooth muscles. *John F. Perkins, Jr. and Charles H.*

Nicholas (introduced by R. W. Gerard). Dept. Physiology, Univ. of Chicago.

7. Viscosity of muscle protoplasm. *Peter Rieser (introduced by L. V. Heilbrunn). Zoological Lab., Univ. of Pennsylvania, Philadelphia.*
8. Effect of 'neurotripsy' on the partially denervated muscle of the dog. *A. J. Kosman and J. N. Frederick (introduced by J. S. Gray). Dept. Physiology, Northwestern Univ. Medical School, Chicago.*
9. An intact mammalian muscle preparation for use in studies on respiratory metabolism. *Shirley A. Johnson* and Kenneth C. Fisher. Univ. of Toronto, Canada.*
10. Characteristics of the thigh muscles with a double action. *J. E. Markee and Maude Williams.* Dept. Anatomy, Duke Univ. School of Medicine, Durham, and Div. of Physiology, Women's College of Univ. of North Carolina, Greensboro.*
11. Influence of work on the amount of k-strophanthoside stored by muscle. *E. C. del Pozo, G. Anguiano* and E. G. Pardo.* Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F.*
12. Influence of hesperidin methyl chalcone on the action of cardiac, striated and smooth muscles. *W. John Kenfield and David F. Bohr (introduced by Robert Gesell). Dept. Physiology, Univ. of Michigan, Ann Arbor.*

FRIDAY, APRIL 22, 1:30 P.M.

Blood Pressure

FOUNTAIN BALLROOM

1. Effect of adrenalectomy on renal VEM system and hypertension. *Benjamin W. Zweifach and Ephraim Shorr. Dept. Medicine, Cornell Univ. Medical College, and New York Hospital, New York City.*
2. Biological fractionation of humoral VEM and VDM in renal hypertension (dogs). *Ephraim Shorr and Benjamin W. Zweifach. Dept. Medicine, Cornell Univ. Medical College, and New York Hospital, New York City.*
3. Effects of breathing CO_2 on blood pressure and renal circulation in normotensive and hypertensive subjects. *W. J. Little, J. W. Avera and S. W. Hoobler (introduced by M. H. Seevers). Dept. Medicine, Univ. of Michigan Hospital, Ann Arbor.*
4. Persistence of hypertension after removal of the causative ischemic kidney. *Jack Flasher* and Douglas R. Drury. Dept. Physiology, Univ. of Southern California School of Medicine, Los Angeles.*
5. Purification and possible histochemical localization of renin. *John Marshall* and G. E. Wakerlin. Depts. Physiology and Pathology, Univ. of Illinois College of Medicine, Chicago.*
6. Effect of crude renal extracts and purified renin in experimental malignant renal hypertension. *R. O. Burns, Jr.,* W. H. Jasper* and G. E. Wakerlin. Dept. Physiology, Univ. of Illinois College of Medicine, Chicago.*
7. Treatment and prophylaxis of experimental renal hypertension with renal extracts. *G. E. Wakerlin, J. E. Bourque,* John Marshall,* R. O. Burns,* Bess G. Osgood* and Lionel Schour.* Dept. Physiology, Univ. of Illinois College of Medicine, Chicago.*
8. Changes in blood pressure and renal function in hypertensive dogs following abscess production. *J. Stamler,* S. Rodbard and L. N. Katz. Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago.*
9. Treatment and prophylaxis of experimental hypertension with amines. *E. A. Ohler,* G. E. Wakerlin and G. A. Alles.* Dept. Physiology, Univ. of Illinois College of Medicine, Chicago.*
10. Control of early hypertension with dihydrogenated ergot derivatives. *W. G. Kubicek, F. J. Kottke and Mildred E. Olson.* Dept. Physical Medicine, Univ. of Minnesota, Minneapolis.*
11. Rapid production of experimental atherosclerosis by kidney damage in the presence of hypercholesterolemia. *Campbell Moses, Elizabeth R. Thornton* and Grace M. Longabaugh.* Addison H. Gibson Lab., Univ. of Pittsburgh School of Medicine, Penna.*
12. Effect of choline and inositol on hypercholesterolemia and hyperlipemia in the cholesterol-fed chick. *C. Bolene,* J. Stamler,* R. Harris* and L. N. Katz. Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago.*

FRIDAY, APRIL 22, 1:30 P.M.

Tissue Fluids; Blood Elements

CONSISTORY PARLOR

1. Arterial and venous plasma K levels in man. *D. P. Earle, S. J. Farber* and J. D. Alexander.* Dept. Medicine, New York Univ. College of Medicine, New York City.*
2. Na and K depletion in rats by means of cation exchange resins mixed with food. *J. M. Crismon, Dept. Physiology, Stanford Univ. School of Medicine, Calif.*
3. Electrolyte metabolism following massive glucose, mannitol, urea infusions in normal

subjects during hydration and dehydration. *Donald W. Seldin and Robert Tarail (introduced by Donald H. Barron). Dept. Internal Medicine, Yale Univ. School of Medicine, New Haven.*

4. Stimulation of sympathetic nerves and capillary filtration. *Esther Hardenbergh and James V. Maloney, Jr. (introduced by William H. Forbes). Dept. Physiology, Harvard School of Public Health, Boston.*
5. Capillary passage of fluorescein in rabbit skin. *Alfred A. Schiller. Dept. Physiology, Univ. of Illinois College of Medicine, Chicago.*
6. Liberation of histamine by epinephrine. *P. Wheeler* and F. R. Goetzl. Institute of Medical Research, The Permanente Foundation, Oakland, Calif.*
7. Kinetics of cholinesterase of erythrocytes in pernicious and other anemias. *Jean Captain Sabine (introduced by Charles I. Wright). Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.*
8. Polyphenoloxidase activity of blood serum. *Bernard Shacter (introduced by Michael B. Shimkin). Lab. of Exptl. Oncology, Univ. of California Medical School, San Francisco, and the National Cancer Institute, National Institutes of Health, Bethesda, Md.*
9. Permeability of rabbit leucocytes to Na and K. *D. L. Wilson* and J. F. Manery. Dept. Biochemistry, Univ. of Toronto, Canada.*
10. Spectral deviations found in hemolyzed erythrocytes of rabbits fed a purified diet. *S. S. Spicer, J. G. Wooley and Ariel M. Clark (introduced by William J. Bowen). Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.*
11. Anemia-producing properties of hexahomoserine and antagonizing effect of lysine. *Edouard Pagé and R. Gingras.* Depts. Nutrition and Biochemistry, Medical School, Laval Univ., Quebec, Canada.*
12. Physiological, pharmacological and biochemical studies on blood of animals in rage. *David I. Macht and Thomas Hoffmaster.* Dept. Pharmacology, Laboratories of Sinai Hospital, Baltimore.*

FRIDAY, APRIL 22, 1:30 P.M.

CHO and Fat Metabolism

LODGE ROOM C

1. Effects of the 'hyperglycemic factor' found in insulin preparations. *H. F. Weisberg,* R. Caren,* B. Huddlestun* and R. Levine. Dept. Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago.*
2. Response to intravenous injection of small quantities of insulin. *John A. Nuetzel* and Norman S. Olsen. Dept. Internal Medicine and Oscar Johnson Institute, Washington Univ. School of Medicine, and Barnes Hospital, St. Louis.*
3. Nature of the action of insulin on the level of serum inorganic phosphate. *R. Levine, S. D. Loube* and H. F. Weisberg.* Dept. Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago.*
5. Turnover rates of acid-soluble phosphorus compounds of liver. *Jacob Sacks and Shepherd Stigman.* Biology Dept., Brookhaven National Laboratory, Upton, L. I., N. Y.*
6. Conversion of propionate carbon to liver glycogen in the intact rat. *Victor Lorber, Margaret Cook* and Julien Bordeaux.* Dept. Biochemistry, Western Reserve Univ. School of Medicine, Cleveland.*
7. Influence of niacin on the excretion of ketone bodies in normal fasted rats. *R. G. Janes and J. Brady.* Dept. Anatomy, State Univ. of Iowa, Iowa City.*
8. Fat metabolism in Alaskan eskimos. *Victor E. Levine and Charles G. Wilber. Arctic Research Laboratory, O.N.R., Point Barrow, Alaska.*
9. Lipoprotein in dog serum. *Lena A. Lewis and Irvine H. Page. Research Division and Bunts Institute, Cleveland Clinic Foundation, Ohio.*
10. Lipid metabolism in Arctic Alaskan birds. *X. J. Musacchia (introduced by Charles G. Wilber). Biological Lab., Fordham Univ., New York City.*
11. Complete parenteral alimentation. *H. C. Meng (introduced by Howard J. Curtis). Dept. Physiology, Vanderbilt Univ. Medical School, Nashville.*

FRIDAY, APRIL 22, 1:30 P.M.

Gastro-Enterology

LODGE ROOM D

1. Trial of enterogastrone in Mann-Williamson dogs. *Frank E. Visscher and Stanley C. Lyster.* Dept. Pharmacology and Endocrinology, Laboratories of the Upjohn Company, Kalamazoo, Mich.*
2. Hormones and hormone-like substances in the treatment of (experimental) peptic ulcer. *David J. Sandweiss and Harry C. Saltzstein.* Harper Hospital and Dept. Surgery, Wayne Univ. College of Medicine, Detroit.*

3. Possible relationship between cinchophen ulceration of the stomach or duodenum and the choleretic response to cinchophen. *D. F. Magee,* K. S. Kim* and A. C. Ivy. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
4. Early stages of gastric surface epithelization. *Rhoda Grant (introduced by A. C. Ivy). Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
5. Influence of intestinal motility on intestinal blood flow. *M. Sidky Mohamed* and John W. Bean. Dept. Physiology, Univ. of Michigan, Ann Arbor.*
6. Quiescence preceding peristaltic constriction in the ileum. *Hugo Krueger. Dept. Zoology, Oregon State College, Corvallis.* (Pharmacol.).
7. Intestinal motility and blood flow as influenced by arterial pressure. *John W. Bean and M. Sidky Mohamed.* Dept. Physiology, Univ. of Michigan, Ann Arbor.*
8. Effect of cinchophen on the secretory activity of Brunner's glands. *K. Hartiala* and M. I. Grossman. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
9. Effects of pancreatic duct ligation upon the action of secretin and pancreozymin in rabbits. *C. C. Wang,* K. J. Wang* and A. C. Ivy. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
10. Pancreatic secretion in response to mechanical stimulation of the duodenum. *J. M. Waldron,* J. E. Thomas and L. P. Tkacz.* Dept. Physiology, Jefferson Medical College, Philadelphia.*
11. Statistical studies of secretin responses in individuals without pancreatic disease. *David A. Dreiling* and Franklin Hollander. Gastroenterology Research Lab., Mount Sinai Hospital, New York City.*
12. Alcohol and sucrose in the production of liver damage in rats. *C. H. Best, W. Stanley Hartroft,* C. C. Lucas and Jessie H. Ridout.* Banting and Best Dept. Medical Research, Univ. of Toronto, Canada.*
13. Effect of diets containing certain tissues on liver regeneration in the rat. *E. Newman* and A. C. Ivy. Dept. Clinical Science, Univ. of Illinois College of Medicine, Chicago.*

FRIDAY, APRIL 22, 1:30 P.M.

Muscle; General Physiology

ROOM 4

1. Initial effects of K on mechanical responses of skeletal muscle. *Alexander Sandow and Arthur J. Kahn.* Washington Square College of Arts and Sciences, New York Univ., New York City.*
2. Effect of temperature on response of rat muscle to indirect repetitive stimulation. *Sheppard M. Walker. Dept. Physiology, Washington Univ. School of Medicine, St. Louis.*
3. Factors influencing the contractile process. *Saul R. Korey (introduced by David Nachmansohn). Dept. Neurology, Columbia Univ. College of Physicians and Surgeons, New York City.*
4. Insulin and the rate of oxygen consumption by frog muscle. *Desmond R. H. Gourley* and Kenneth C. Fisher, Univ. of Toronto, Canada.*
5. Electrophoretic study of the water soluble proteins of normal and atrophied muscles. *E. Fischer, N. J. Copenhaver,* B. Galeski* and K. W. Ryland.* Baruch Center of Physical Medicine, Medical College of Virginia, Richmond.*
6. Specific resistance of regenerating Dugesia in different media and electrical work of polarity control. *Gordon Marsh and Jean Dimmitt.* Zoological Labs., State Univ. of Iowa, Iowa City.*
7. Potential gradient vs. current density in electrical control of axial polarity in regenerating Dugesia tigrina. *Jean Dimmitt (introduced by Gordon March). Zoological Labs., State Univ. of Iowa, Iowa City.*
8. Osmophilia in relation to alkaline phosphatase activity in the chloride cell (gill) of Fundulus Heteroclitus. *D. Eugene Copeland (introduced by C. M. Williams). Arnold Biological Lab., Brown Univ., Providence.*
9. Analysis of mosquito tissues for Na and K and development of a physiological salt solution. *Sarah Bradford* and Robert W. Ramsey. Dept. Physiology and Pharmacology, Medical College of Virginia, Richmond.*
10. Active salt transport and its regulation in the isolated frog skin. *Ernst G. Huf (introduced by Ernst Fischer). Baruch Center of Physical Medicine and Dept. Physiology, Medical College of Virginia, Richmond.*
11. Isotopic studies of K exchange rates between cellular elements and plasma of human and canine blood *in vitro*. *C. W. Sheppard and W. R. Martin (introduced by Alexander Hollaender). Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.*
12. Adsorption and fluorescence characteristics of crustacean sinus gland extracts. *Eloise Kuntz (introduced by Irwin W. Sizer). Arnold Biological Lab., Brown Univ., Providence.*

Papers Read by Title

1. Adrenalectomy and renin proteinuria in the rat. *T. Addis and Robert I. Boyd.* Dept. Medicine, Stanford Univ. Medical School, San Francisco.*
2. Effect of aging on O_2 consumption of non-nucleated erythrocytes. *Luis Angelone* and Clifford A. Angerer. Dept. Physiology, Ohio State Univ., Columbus.*
3. Hyperplasia of chromaffin material in sympathetic paraganglia of adrenalectomized rats. *H. Bacchus,* A. Dury and D. B. Young.* Depts. Physiology and Zoology, George Washington Univ., Washington, D. C.*
4. Quantitative method of estimating physiological effects of deep-breathing on the electroencephalogram. *T. C. Barnes and Marie D. Amoroso.* Dept. Electroencephalography, Hahnemann Medical College and Hospital of Philadelphia.*
5. Renal function in untreated adrenalectomized rats. *W. R. Boss,* James H. Birnie* and Robert Gaunt. Dept. Zoology, Syracuse Univ., Syracuse, N. Y.*
6. A method for analyzing dog muscles for myoglobin. *William J. Bowen. Lab. of Physical Biology, Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.*
7. Gravity and applied accelerations. *S. W. Britton and O. B. Murray, Jr.* Physiological Laboratory, Univ. of Virginia, Charlottesville.*
8. Analysis of the mechanism of diurnal rhythmicity in crustacea. *Frank A. Brown, Jr. and H. Marguerite Webb.* Dept. Zoology, Northwestern Univ., Evanston, Ill., and Marine Biological Lab., Woods Hole, Mass.*
9. Effects of K and lanatoside-C on the failing heart in heart-lung preparations. *Sandor G. Burstein,* Leslie L. Bennett, Francis E. Payne* and James Hopper, Jr.* Divisions of Physiology and Medicine, Univ. of Calif., Berkeley and San Francisco.*
10. Studies on bound acetylcholine. *Daniel J. Cavanaugh and Julian M. Tobias. Dept. Physiology and Toxicity Laboratory, Univ. of Chicago.*
11. Effect of substrate concentration on the luminescent reaction of Cypridina luciferin and luciferase. *Aurin M. Chase. Physiological Lab., Princeton Univ., N. J., and Marine Biological Lab., Woods Hole, Mass.*
12. Proteolytic activity in cerebro-spinal fluid. *George Constant,* Wiktor W. Nowinski, Robert B. White* and Joy M. Hart.* Tissue Culture Lab. and Dept. Neurology and Psychiatry, Univ. of Texas Medical Branch, Galveston.*
13. Ferretin: effect on blood pressure and pressor response in rats. *A. C. Corcoran, G. Masson and C. Schaffenburg.* Research Division and Bunts Institute of Cleveland Clinic Foundation, Ohio.*
14. Attempted blockade of adrenotrophic mechanism of the pituitary in starvation. *Savino A. D'Angelo. Dept. Biology, Washington Square College of Arts and Science, New York Univ., New York City.*
15. Protein utilization in hypophysectomized dogs. *R. C. de Bodo, S. P. Kiang* and I. H. Slater.* Dept. Pharmacology, New York Univ. College of Medicine, New York City.*
16. Carbohydrate utilization and lactate formation in the chicken retina. *H. F. Diermeier and V. F. Lindeman (introduced by Robert Gaunt). Dept. Zoology, Syracuse Univ., N. Y.*
17. Significance of activation of prothrombin in serum. *John H. Ferguson. Dept. Physiology, Univ. of North Carolina, Chapel Hill.*
18. Sensibility of man to light anoxia. *P. E. Fiset* and L. P. Dugal. Dept. d'Acclimatation, Institut de Biologie Humaine, Univ. Laval, Québec, Canada.*
19. Electrolyte and protein changes during acute pneumococcal infection in rats. *Charles L. Fox, Jr. and Gilbert Ashwell (introduced by Alfred Gilman). Dept. Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.*
20. Effect of Eck fistula formation and meat intoxication on serum phosphatase and dye clearance of adult dogs. *Smith Freeman. Dept. Experimental Medicine, Northwestern Univ. Medical School, Chicago.*
21. Inactivation or removal of insulin by the liver. *A. Friedman,* H. F. Weisberg* and R. Levine. Dept. Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago.*
22. Emotional state on acquired and inborn reactions: satiation on cardiac conditional reflexes and unconditional reflexes to food. *W. Horsley Gantt. Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Univ., Baltimore, Md.*
23. Physical and chemical properties of emulsifying agents in urine. *Josephine B. Garst* and Harry B. Friedgood. Dept. Medicine, School of Medicine, Univ. of California at Los Angeles; California Institute for Cancer Research.*
24. Cholinesterase activity of intact nerve; DFP inhibition. *R. W. Gerard, B. Libet and D. Cavanaugh. Dept. Physiology, Univ. of Chicago, Ill., and Marine Biological Lab., Woods Hole, Mass.*

25. Effects of adrenal resection on hypertension and diabetes. *D. M. Green, J. N. Nelson* and G. A. Dodds.* Dept. Medicine, Univ. of Washington School of Medicine, Seattle.*
26. Light flux constancy at intensity limin of the eye for total receptor cross-sectional areas. *Charles Haig. Dept. Physiology and Biochemistry, New York Medical College, New York City.*
27. Effects of lanatoside C on ectopic ventricular activity following coronary occlusion. *A. Sidney Harris and Robert H. Kokernot.* Baylor Univ. College of Medicine, Houston, Texas.*
28. Uptake of radiocalcium by the skeleton. *Harold E. Harrison and Helen C. Harrison (introduced by Nathan W. Shock). Dept. Pediatrics, Johns Hopkins Univ. Medical School, and Pediatric Division, Baltimore City Hospitals, Md.*
29. Consciousness and reflex potentialities of dogs during immersion hypothermia. *H. O. Haterius and A. H. Hegnauer. Dept. Physiology, Boston Univ. School of Medicine, Mass.*
30. Temperature gradients in dogs in immersion hypothermia. *A. H. Hegnauer. Dept. Physiology, Boston Univ. School of Medicine, Mass.*

30a. Adrenal cortical function and effect of adrenocorticotropin in myasthenia gravis. *Leon Hellman (introduced by R. E. Weston). Medical Division, Montefiore Hospital, New York City.*

31. Mechanism and management of edema. *George R. Herrmann, John W. Chriss,* E. H. Schwab* and Paul M. Sims.* Univ. of Texas Medical Branch, Galveston.*
32. Effect of Tridione on convulsions caused by di-isopropyl fluorophosphate (DFP). *H. E. Himwich, C. F. Essig* and J. L. Hampson.* Medical Division, Army Chemical Center, Md.*
33. Failure of topical Ca and Mg to prevent eugenol-induced desquamation of gastric mucous epithelium. *Franklin Hollander and Shirley D. Kraus.* Gastroenterology Research Laboratory, Mount Sinai Hospital, New York City.*
34. Disappearance of mannitol and PAH from plasma of bilaterally nephrectomized dogs. *C. Riley Houck. Division of Physiology, Univ. of Tennessee, Memphis.*
35. Antihemolytic action of a hemolytic agent. *M. H. Jacobs, Marian Willis LeFevre* and Carolyn Stout.* Dept. Physiology, Univ. of Pennsylvania, Philadelphia.*
36. Site of action of Myanesin (Tolserol) in the central nervous system. *B. R. Kaada (introduced by John F. Fulton). Lab. of Physiology, Yale Univ. School of Medicine, New Haven.*
37. Isolation of legcoproporphyrin. *Heinrich Klüver. Division of Biological Sciences, Univ. of Chicago.*
38. Skin resistance patterns associated with visceral disease. *Irvin M. Korr. Kirksville College of Osteopathy & Surgery, Mo.*
39. Effect of handometer exercise on normal grip strength. *Eleanor M. Larsen (introduced by W. J. Meek). Dept. Physiology, Univ. of Wisconsin Medical School, Madison.*
40. Biological studies with 1-methylestrone. *J. H. Leathem and Mary Lee Clary.* Rutgers Univ., New Brunswick, N. J., and Ciba Pharmaceutical Products, Summit, N. J.*
41. Effect of temperature on membrane potential of frog sartorius muscle. *G. Ling and J. W. Woodbury (introduced by R. W. Gerard). Dept. Physiology, Univ. of Chicago, Ill., and Dept. Physiology, Univ. of Utah, Salt Lake City.*
42. Experimental studies with psoriatic blood. *David I. Macht. Dept. Pharmacology, Laboratories of Sinai Hospital, Baltimore.*
43. Presence of multiple cortical foci in primary sensory projection systems of the cat. *Wade H. Marshall. National Institutes of Health, Bethesda, Md.*
44. Effect of low barometric pressure on the brains of five vertebrates. *Bernard Metz (introduced by Charles G. Wilber). Biological Laboratory, Fordham Univ., New York City.*
45. Influence of cardiac sympathetic nerves on cardiopressor action of the vagus nerve. *S. Middleton and J. Talesnik (introduced by Harold C. Wiggers). Physiological Institute, Univ. of Chile, Santiago.*
46. Effect of nephrotoxic sera on clearance of urea, creatinine and para-aminohippurate in dogs. *Campbell Moses and Elizabeth R. Thornton.* Addison H. Gibson Lab., Univ. of Pittsburgh School of Medicine.*
47. Cardiac effects of aliphatic sympathomimetic amines in dogs under cyclopropane anesthesia. *Quill R. Murphy* and Walter J. Meek. Dept. Physiology, Univ. of Wisconsin Medical School, Madison.*
49. Graphic measurement of arterial pressure in the intact rat. *F. Olmstead and O. Glasser (introduced by A. C. Corcoran). Research Division, Cleveland Clinic Foundation and Bunts Institute, Ohio.*

50. Diuretic effect of adrenal cortical hormones in mildly hydrated and dehydrated animals. *C. M. Osborn and W. J. Eversole (introduced by Robert Gaunt). Dept. Zoology, Syracuse Univ., N. Y.*

51. Oxygen consumption and water balance in the X-irradiated frog. *Harvey M. Patt, Marguerite N. Swift* and Ella B. Tyree.* Biology Division, Argonne National Laboratory, Chicago.*

53. Effect of estrogen and relaxin on connective tissue of the pubic symphysis. *E. Perl* and H. R. Catchpole. Dept. Pathology, College of Medicine, Univ. of Illinois, Chicago.*

54. Effect of protein in the diet on certain aspects of renal function. *Theodore N. Pullman,* Alf S. Alving and Milton Landowne. Dept. Medicine, Univ. of Chicago.*

55. Multiple avitaminosis-like syndrome produced by adenine. *Sigwin B. Raska. Dept. Pharmacology, Univ. of Mississippi School of Medicine, University.*

56. Age and kidney renin in rabbits. *G. Carl Raú (introduced by M. M. Ellis). Dept. Physiology and Pharmacology, Medical School, Univ. of Missouri, Columbia.*

57. Relation of pituitary to enterogastrone and urogastrone. *D. J. Sandweiss, J. Kaulbersz, T. L. Patterson and H. C. Saltzstein.* Depts. Physiology and Surgery, Wayne Univ. College of Medicine, and Harper Hospital, Detroit.*

58. Circulation effects of electrophrenic respiration and positive pressure breathing during high spinal anesthesia. *Stanley J. Sarnoff, James V. Maloney, Jr. and James L. Whittenberger (introduced by William H. Forbes). Dept. Physiology, Harvard School of Public Health, Boston.*

59. Anaphylactic release of histamine and heparin from isolated dog liver. *A. E. Scroggie* and L. B. Jaques. Dept. Physiology, Univ. of Saskatchewan, Saskatoon, Canada.*

60. Diurnal variations in glomerular activity in normal man and in patients with congestive heart failure. *Jonas H. Sirota, David S. Baldwin and Herman Villarreal (introduced by Homer W. Smith). Depts. Physiology and Medicine, New York Univ. College of Medicine, New York City.*

61. Augmentation by catechol and P-cresol of oxidation of proteins by mushroom tyrosinase. *Irwin W. Sizer and Clyde O. Brindley.* Dept. Biology, Massachusetts Institute of Technology, Cambridge.*

62. Rate of elimination of sulfadiazine from blood in the newborn. *Franklin F. Snyder. Depts. Obstetrics and Anatomy, Harvard Medical School, Boston.*

63. Electroshock studies: convulsant effect of microwaves; changes of current during stimulation. *E. A. Spiegel, P. H. Wilcox* and A. J. Lee.* Dept. Experimental Neurology. Temple Univ. School of Medicine, Philadelphia, Penna., and Traverse City State Hospital, Mich.*

64. Effect of cold adaptation on highest and lowest temperatures at which turtle heart continues beating. *D. E. Stullken,* F. M. White* and W. A. Hiestand. Lab. of Animal Physiology, Purdue Univ., Lafayette, Ind.*

65. Effects of various concentrations of potassium cyanide on Pelomyxa carolinensis. *Louis A. Susca* and Charles G. Wilber. Biological Lab., Fordham Univ., New York City.*

66. Free amino acids in the plasma of Eck fistula, meat intoxicated and hepatectomized dogs. *Muriel H. Svec (introduced by Smith Freeman). Dept. Experimental Medicine, Northwestern Univ. Medical School, Chicago.*

67. Measurement of renal plasma flow in man by the slope method without urine analysis. *Hall S. Tacket (introduced by C. Riley Houck). Divisions of Medicine and Physiology, Univ. of Tennessee, Memphis.*

68. Origin of renal vasoconstriction in tourniquet shock. *R. D. Taylor and Irvine H. Page. Research Division and Bunts Institute, Cleveland Clinic Foundation, Ohio.*

70. Amino acids and neuro-muscular function. *Clara Torda and Harold G. Wolff. New York Hospital and Depts. Medicine (Neurology) and Psychiatry, Cornell Univ. Medical College, New York City.*

71. Thrombin formation in presence of proteolytic enzymes and platelets. *Burton L. Travis (introduced by John H. Ferguson). Dept. Physiology, Univ. of North Carolina, Chapel Hill.*

72. Renin concentration of the kidney in experimental renal hypertension. *G. E. Wakerlin, John Marshall,* R. O. Burns* and Bess G. Osgood.* Dept. Physiology, Univ. of Illinois College of Medicine, Chicago.*

73. Testing validity of ashing and digestion procedures by isotope dilution. *Walter S. Wilde. Dept. Physiology, Tulane Univ., New Orleans.*

74. Hepatic vital staining and dye excretion in

CCl_4-treated mice. *W. Lane Williams. Dept. Anatomy, Univ. of Minnesota, Minneapolis.*

75. Treatment of peptic ulcer with desiccated defatted whole duodenum: report of failure. *C. W. Wirts* and M. H. F. Friedman. Jefferson Medical College, Philadelphia.*

77. Anti-stiffness factor in relation to hearing in guinea pigs. *Rosalind Wulzen and Alice B. Plympton.* Dept. Zoology, Oregon State College, Corvallis.*

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INC.

Fortieth Annual Meeting

An asterisk following an author's name denotes "by invitation"

TUESDAY, APRIL 19, 9:00 A.M.

Folic Acid and Vitamin B_{12}

COMMANDERY ASYLUM

1. Pteroylglutamic acid deficiency in the dog as produced by 'x-methyl' pteroylglutamic acid. *A. L. Franklin*, T. H. Jukes, E. L. R. Stokstad, and M. Belt*. Lederle Labs. Div., American Cyanamid Co. Pearl River, N. Y.*
2. Response of pteroylglutamic acid (PGA) depleted rats to supplementation with natural products. *Conrado F. Asenjo. Dept. of Chemistry and Nutrition, School of Tropical Medicine, San Juan, Puerto Rico.*
3. Biochemical studies on the livers of chicks receiving graded levels of pteroylglutamic acid. *John R. Totter, William Martindale*, Marion McKee*, Cecilia K. Keith*, and Paul L. Day. Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock.*
4. Influence of xanthopterin on the folic acid content of liver, *in vitro*. *Arnold D. Welch, Evelyn M. Nelson*, and Marilyn F. Wilson*. Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland (Pharmacol.).*
5. Factors concerned in the liberation of pteroylglutamic (folic) acid from its heptaglutamate by chick liver. *M. L. Scott*, C. H. Hill*, and L. C. Norris. Cornell Univ., Ithaca, N. Y.*
6. Relationship of purines, folic acid, and other principles in the nutrition of *Leuconostoc citrovorum* 8081. *H. E. Sauberlich (introduced by W. D. Salmon). Lab. of Animal Nutrition, Alabama Polytechnic Inst., Auburn.*
7. Pteridines and Xanthine oxidase activity. *Edwin G. Krebs* and Earl R. Norris. Dept. of Biochemistry, Univ. of Washington, Seattle.*
8. Influence of pteroylglutamic acid on glycine and on porphyrin metabolism. *Cecilia K. Keith* and John R. Totter. Dept of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock.*
9. Effect of folic acid, liver extract and vitamin B_{12} on hemoglobin regeneration in chicks. *C. A. Nichol*, A. E. Harper*, and L. S. Dietrich*, and C. A. Elvehjem. Dept of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.*
10. Effect of pteridines on hemapoiesis and cell proliferation. *Earl R. Norris and John J. Majnarich*. Dept. of Biochemistry, Univ. of Washington, Seattle.*
11. Microbiological assay of vitamin B_{12} by *lactobacillus leichmannii*. *E. L. R. Stokstad, A. C. Dornbush*, A. L. Franklin*, C. E. Hoffman*, B. L. Hutchings and T. H. Jukes. Lederle Labs. Div., American Cyanamid Co., Pearl River. N. Y.*
12. Paper chromatography of vitamin B_{12} and related bacterial growth factors. *Walter A. Winsten and Edward Eigen (introduced by Philip B. Hawk). Food Research Labs., Inc., Long Island City, N. Y.*

TUESDAY, APRIL 19, 9:00 A.M.

Amino Acid Metabolism I

LODGE ROOM E

1. Function of the system, glutamic acid-glutamine, in brain metabolism. *Heinrich Waelsch, Paula Schwerin*, and S. P. Bessman*. Depts. of Biochemistry, New York State Psychiatric Inst. and Columbia Univ., New York City.*
2. Effects of anterior pituitary growth hormone preparations on the glutamine-glutaminase system. *Paul D. Bartlett (introduced by Oliver H. Gaebler). Edsel B. Ford Inst. for Medical Research, Henry Ford Hospital, Detroit.*
3. Intermediary metabolism of phenylalanine labeled with C^{14}. *Bernard Schepartz* and Samuel Gurin. Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
4. Studies on copper-containing oxidases in higher plants. *M. L. Rothchild and Robert MacVicar (introduced by V. G. Heller). Depts. of Chemistry, Oklahoma A. and M. College and Agricultural Chemistry Research, Oklahoma Agricultural Experiment Station, Stillwater.*
5. A microbiological determination of D-phenylalanine. *Ernest Borek and Heinrich Waelsch. Depts. of Biochemistry, New York State Psychiatric Inst. and Columbia Univ., New York City.*

6. Quantitative separation and determination of small amounts of histidine and tyrosine employing paper chromatography. *Diana Bolling*, Herbert A. Sober*, and Richard J. Block. Dept. of Biochemistry and Physiology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.*
7. Mammalian tyrosinase. Action on compounds structurally related to tyrosine and dihydroxyphenylalanine. *A. Bunsen Lerner*, T. B. Fitzpatrick*, and W. H. Summerson. Biochemistry Section, Medical Div., Army Chemical Center, Md.*
8. Purification of diamine oxidase (histaminase). *Herbert Tabor. Experimental Biology and Medicine Inst., National Inst. of Health, Bethesda, Md. (Pharmacol.).*
9. Inhibitors of the L-amino acid oxidase of snake venoms. *Thomas P. Singer and Edna B. Kearney*. Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland.*
10. Effect of amino acid administration on amino acid oxidase. *R. N. Cagan*, J. L. Gray*, and H. Jensen. Medical Dept. Field Research Lab., Fort Knox, Ky.*
11. Flavoenzyme catalysis. Sulfydryl character of the D-amino acid oxidase. *W. R. Frisell*, E. C. Weinbach*, R. C. Cowgill*, and Leslie Hellerman. Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore.*
12. Nitrogenous metabolism of the earthworm (Lumbricus terrestris). *Stanley Cohen* and Howard B. Lewis. Dept. of Biological Chemistry, Medical School, Univ. of Michigan, Ann Arbor.*

TUESDAY, APRIL 19, 9:00 A.M.

Lipid Chemistry

ROOM 5

1. A new P-free and S-free brain lipide. *J. Folch and S. Arsove*. McLean Hospital, Waverly Mass. and Harvard Medical School, Boston.*
2. Further studies on the lipids of peripheral nerve during Wallerian degeneration. *A. C. Johnson*, A. R. McNabb*, and R. J. Rossiter, Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*
3. Composition of brain lipids in male and female rats. *Warren M. Sperry, Florence C. Brand*, and Merrill Webb*. Dept. of Biochemistry, New York State Psychiatric Inst., New York City.*
4. Inositol lipids of soybean. *Herbert E. Carter, Walter D. Celmer*, Mack H. McCormick*, Clifford O. Nyman*, and Frances W. Saunders*. Div. of Biochemistry, Noyes Lab. of Chemistry, Urbana, Ill.*
5. Investigation of phospholipid composition in minute amounts. *Celia Levine* and Erwin Chargaff. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.*
6. Quantitative determination of glycerylphosphorycholine (GPC) in tissues. *Gerhard Schmidt, L. Hecht*, N. Strickler*, and S. J. Thannhauser. Tufts College Medical School, Boston.*
7. Micro molecular distillation of fatty acids. *Alfred E. Koehler and Elsie Hill*. Santa Barbara Cottage Hospital and Sansum Clinic Research Foundation, Santa Barbara, Calif.*
8. Cholesterol studies on lyophilized chicken serum. *J. C. Forbes, G. H. L. Dillard*. Dept. of Biochemistry, Medical College of Virginia, Richmond.*
9. Detection of 7-keto-cholesterol in blood plasma. *H. B. Collier and R. H. Cox*. Dept. of Biochemistry and College of Pharmacy, Univ. of Saskatchewan, Saskatoon, Sask.*
10. On the rate and mechanism of the *Liebermann-Burchard* reaction on various derivatives of cholesterol. *Herbert McKennis, Jr., Joseph Y. Thomas*, and J. C. Forbes. Lab. for Surgical Research, Dept. of Biochemistry, Medical College of Virginia, Richmond; Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore.*
11. Demonstration of specificity of a simplified method for the determination of total cholesterol. *Forrest E. Kendall, Liese Lewis Abell*, Betty B. Levy*, J. Murray Steel*, and Bernard B. Brodie. Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ.; Depts. of Medicine and Biochemistry, New York Univ. College of Medicine; New York Univ. and Columbia Research Services, Goldwater Memorial Hospital, New York City.*

TUESDAY, APRIL 19, 1:30 P.M.

Acetate Metabolism

COMMANDERY ASYLUM

1. Acetyl precursor in aromatic amine acetylation in pigeon liver extracts. *Nathan O. Kaplan and Morris Soodak (introduced by Fritz Lipmann). Biochemical Research Lab. Massachusetts General Hospital and Dept. of Biological Chemistry, Harvard Medical School, Boston.*
2. Non participation of acetyl phosphate in the fixation of formate into pyruvate. *Harold Strecker* and Harland G. Wood. Dept. of Bio-*

chemistry, Western Reserve Univ. School of Medicine, Cleveland.

3. Acetate oxidation by a washed kidney cortex preparation. *George Kalnitsky. Dept. of Biochemistry, State Univ. of Iowa, Iowa City.*
4. Acetoacetate production from hexanoic and other six carbon acids by rat liver preparations. *Robert F. Witter* and Elmer Stotz. Dept. of Biochemistry, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.*
5. *In vitro* utilization of C^{14} labeled acetate and pyruvate by rat diaphragm muscle. *Claude A. Villee, Olof H. Pearson*, and A. Baird Hastings. Dept. of Biological Chemistry, Harvard Medical School, Boston.*
6. Metabolism of isovaleric acid studied with isotopic carbon. *Irving Zabin* and Konrad Bloch. Dept. of Biochemistry and Inst. of Radiobiology and Biophysics, Univ. of Chicago.*
7. Metabolism of 2,4- and 3,5-diketo acids. *Alton Meister (introduced by Jesse P. Greenstein). National Cancer Inst., National Inst. of Health, Bethesda, Md.*
8. Metabolism of acetone. *T. D. Price* and D. Rittenberg. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.*
9. Studies on the formation of acetoacetic acid from radioactive leucine. *Minor J. Coon, (introduced by D. Wright Wilson). Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
10. Influence of pituitary and adrenal glands on ketone body production by rat liver slices. *Philip K. Bondy* and Alfred E. Wilhelmi. Dept. of Physiological Chemistry, Yale Medical School, New Haven.*
11. Studies on the biological synthesis of cholesterol from acetic acid. *Henry N. Little* and Konrad Bloch. Dept. of Biochemistry and Inst. of Radiobiology and Biophysics, Univ. of Chicago.*
12. Acetate metabolism and ergosterol synthesis in neurospora. *R. C. Ottke*, E. L. Tatum, and S. Simmonds. Dept. of Microbiology, Yale Univ., New Haven, and Dept. of Biology, Stanford Univ., Palo Alto, Calif.*

TUESDAY, APRIL 19, 4:30 P.M.

Biochemistry Business Meeting

COMMANDERY ASYLUM

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

Program on p. 418

TUESDAY, APRIL 19, 1:30 P.M.

Nutrition

LODGE ROOM E

1. Biosynthesis of carotenes in tomatoes. *John W. Porter and Ralph E. Lincoln (introduced by F. W. Quackenbush). Depts of Agricultural Chemistry and Botany and Plant Pathology, Purdue Univ., Lafayette, Ind.*
2. Studies on the conversion of carotene to vitamin A by the rat. *J. G. Bieri (introduced by M. O. Schultze). Div. of Agricultural Biochemistry, Univ. of Minnesota, Minneapolis.*
3. Enzymic reduction of the retinenes to the Vitamins A. *George Wald. Biological Labs. of Harvard Univ., Cambridge, Mass. (Physiol.).*
4. Interplay of light and heat in bleaching rhodopsin. *Robert C. C. St. George (introduced by George Wald). Biological Labs. of Harvard Univ., Cambridge, Mass. (Physiol.).*
5. A biologically-active, reversible oxidation product of alpha-tocopherol. *Paul D. Boyer. Div. of Biochemistry, Univ. of Minnesota, St. Paul.*
6. Hemolytic action of alloxan and alloxan derivatives. *Catharine S. Rose* and Paul Gyorgy. Nutritional Service of the Dept. of Pediatrics and the Gastro-Intestinal Section of the Medical Clinic, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
7. Effect of dietary manganese on the growth of white rat. *Dorsey E. Holtkamp* and Robert M. Hill. Dept. of Biochemistry, Univ. of Colorado Medical Center, Denver.*
8. Dietary manganese and suceptibility to pneumococcal infection. *George H. Hitchings, Elvira A. Falco*, and Marion B. Sherwood*. Wellcome Research Labs., Tuckahoe, N. Y.*
9. Comparative studies in niacin metabolism. *W. A. Perlzweig, Fred Rosen*, I. G. Leder*, and S. Hunter*, and P. B. Pearson. Dept. of Biochemistry, Duke Univ. and A. and M. College of Texas, College Station.*
10. Identification of L-ascorbic acid in the urine of normal and chloretone treated rats. *G. W. Probst* and M. O. Schultze. Div. of Agricultural Biochemistry, Univ. of Minnesota, Minneapolis.*
11. Short-term pyridoxine deprivation in two human subjects. *Louis D. Greenberg, David*

F. Bohr, Hope McGrath*, and James F. Rinehart*. Dept of Pathology, Univ. of California Medical School, San Francisco.*

TUESDAY, APRIL 19, 4:30 P.M.

Biochemistry Business Meeting

COMMANDERY ASYLUM

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

Program on page 418

TUESDAY, APRIL 19, 1:30 P.M.

Physical Chemistry of Proteins

ROOM 5

1. Purification and properties of ovomucoid. *E. Fredericq* and H. F. Deutsch. Depts. of Physical and Physiological Chemistry, Univ. of Wisconsin, Madison.*
2. Isolation and physicochemical characterization of pituitary follicle-stimulating hormone. *Choh Hao Li. Inst. of Experimental Biology, Univ. of California, Berkeley.*
3. Amino acid composition of casein, α-casein and β-casein. *William G. Gordon, William F. Semmett*, and Myron Morris*. Eastern Regional Research Lab., Philadelphia.*
4. Specific volume of α- and β-casein and relationship of specific volume to amino acid composition. *T. L. McMeekin, M. L. Groves* and N. J. Hipp*. Eastern Regional Lab., Philadelphia.*
5. Interaction of 9-aminoacridines with proteins and nucleic acids. *J. Logan Irvin and Elinor Moore Irvin*. Dept. of Physiological Chemistry, Johns Hopkins Univ. School of Medicine, Baltimore.*
6. Deamination of crystalline egg albumin. *Paul Maurer* and Michael Heidelberger. Depts. of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.*
7. Combination of wool protein with strong bases. *Jacinto Steinhardt and Ethel M. Zaiser*. Dept. of Chemistry, Massachusetts Inst. of Technology, Cambridge.*
8. Effects of certain organic anions on amide and peptide linkages. *Granvil C. Kyker and Sanford L. Steelman*. Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill.*
9. Formation of protein or other aggregates by reactions linked in series. *David F. Waugh. Dept. of Biology, Massachusetts Inst. of Technology, Boston.*
10. On the likelihood of specific long range forces in immunologic and enzymatic reactions. *S. J. Singer (introduced by L. Zechmeister). Gates and Vrellin Labs. of Chemistry, California Inst. of Technology, Pasadena.*
11. Effect of pH on the three waves of the protein polarogram. *G. J. Miller (introduced by L. B. Jacques). Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada (Physiol.).*
12. Polarographically determined protein index. Nature of the cobalt-catalyzed reaction of cysteine. *Otto H. Muller. Dept of Physiology, Syracuse Univ. College of Medicine (Physiol.)*

TUESDAY, APRIL 19, 4:30 P.M.

Biochemistry Business Meeting

COMMANDERY ASYLUM

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

Program on page 418

WEDNESDAY, APRIL 20, 9:00 A.M.

Oxidation and Phosphorylation

COMMANDERY ASYLUM

1. Oxidative phosphorylation without deamination in mitochondrial preparations. *Van R. Potter and W. C. Schneider*. McArdle Memorial School, Univ. of Wisconsin, Madison.*
2. Anaerobic phosphorylation due to a coupled oxidation-reduction between α-ketoglutaric acid and α-iminoglutaric acid. *F. Edmund Hunter, Jr. Pharmacology Dept. Washington Univ. Medical School, St. Louis.*
3. Esterification of phosphate coupled to electron transport between dihydrodiphosphopyridine nucleotide and oxygen. *Albert L. Lehninger and Morris E. Friedkin*. Depts.*

of Surgery and Biochemistry, Univ. of Chicago.

4. Incorporation of P^{32} during the operation of the cyclophorase system. *R. Cross, W. Atchley, and H. Albaum (introduced by D. E. Green). Enzyme Lab., College of Physicians and Surgeons, Columbia Univ., New York City.*
5. Phosphate turnover of various nucleotides in respiring liver homogenates. *J. P. Hummel and Olov Lindberg (introduced by H. A. Mattill). Wenner-Gren's Inst., Univ. of Stockholm, Sweden.*
6. Experiments on the mechanism of coupling between respiration and phosphorylation. *W. F. Loomis (introduced by Fritz Lipmann). Biochemical Research Lab., Massachusetts General Hospital and Dept. of Biochemistry, Harvard Medical School, Boston.*
7. A new metabolic regulator in mammalian spermatozoa. *Henry A. Lardy and D. Ghosh*. Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.*
8. Inorganic pyrophosphate in the enzymatic synthesis of DPN. *Arthur Kornberg (introduced by W. H. Sebrell, Jr.). Experimental Biology and Medicine Inst., National Inst. of Health, Bethesda, Md.*
9. Cytochrome *c* reductase (DPN). *Leon A. Heppel (introduced by B. L. Horecker). Experimental Biology and Medicine Inst., National Inst. of Health, Bethesda, Md.*
10. Cytochrome *c* reductase (TPN) from liver. *B. L. Horecker. Experimental Biology and Medicine Inst., National Inst. of Health, Bethesda, Md.*
11. Demonstration of TPN dehydrogenases in the tissues of higher plants. *Eric E. Conn*, Birgit Vennesland, and E. A. Evans, Jr. Dept. of Biochemistry, Univ. of Chicago.*
12. Effects of metabolic inhibitors on membrane potentials. *Norman R. Joseph, Ervin Kaplan*, and C. I. Reed. Univ. of Illinois Chicago Professional Colleges, Chicago.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Growth Factors; Antibiotics

LODGE ROOM E

1. Quantitative paper chromatography of penicillins. I. Use of aqueous solution as developing agent. *Irving R. Hooper, David L. Johnson, Harold D. Taylor (introduced by S. B. Binkley). Research Div., Bristol Labs., Inc., Syracuse.*
2. Quantitative paper chromatography of penicillins. II. Development of buffered strips with organic solvent. *Oliver B. Fardig and Nancy D. Breed (introduced by S. B. Binkley). Research Div., Bristol Labs., Inc., Syracuse.*
3. Studies of growth factors in liver extract using paper partition chromatography. *Garson H. Tishkoff, H. T. Peeler, Alejandro Zaffarone, Henry Tesluk and L. C. Norris (introduced by Elmer H. Stotz). Dept. of Pathology, Dept. of Biochemistry, School of Medicine and Dentistry, Univ. of Rochester; School of Nutrition, Cornell Univ., Ithaca, N. Y.*
4. Isolation of biochemically deficient mutants of bacteria by means of penicillin. *Bernard D. Davis. U. S. Public Health Service, Tuberculosis Research Lab., Cornell Univ. Medical College, New York (Immunol.).*
5. Purification, crystallization and properties of circulin, an antibiotic from the *Bacillus circulans* group. *Stacey F. Howell. Biochemistry Section, V. D. Research Lab., U. S. Public Health Service, Stapleton, Staten Island, N. Y.*
6. Oxygen consumption of 'killer' and 'sensitive' stocks of *Paramecium aurelia*, variety 4. *D. H. Simonsen* and W. J. van Wagtendonk. Dept. of Zoology, Indiana Univ., Bloomington.*
7. Studies on the isolation of the staphylococcus stimulatory factor (s) (SSF). *Nathan H. Sloane* and Ralph W. McKee. Dept. of Biological Chemistry, Harvard Medical School, Boston.*
8. Comparative study of growth factors for some lactic acid bacteria. *Carl M. Lyman and J. M. Prescott*. Dept. of Biochemistry and Nutrition, Agricultural and Mechanical College of Texas, College Station.*
9. Growth-promotion of yeasts by neopyrithiamin. *Jesse C. Rabinowitz* and Esmond E. Snell. Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.*
10. Growth stimulatory factors for the malarial parasite, *Plasmodium knowlesi. Ralph W. McKee and Quentin M. Geiman*. Depts. of Biological Chemistry, Comparative Pathology, Tropical Medicine, Harvard Medical School, Boston.*
11. Bacterial synthesis in the rat of an unidentified growth-promoting factor. *A. M. Hartman*, L. P. Dryden* and C. A. Cary. Div. of Nutrition and Physiology, Bureau of Dairy Industry, U. S. Dept. of Agriculture, Beltsville, Md.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Liver Proteins

ROOM 5

1. Hepato-renal factors in circulatory homeostasis. XXII. Chemical properties of ferritin and apoferritin. *Abraham Mazur and Ephraim Shorr*. Dept. of Medicine, Cornell Univ. Medical College and New York Hospital, New York City.*
2. Fractionation and chemical studies of proteins of rat liver. *Alfred Chanutin, E. C. Gjessing and Stephan Ludewig. Biochemical Lab., Univ. of Virginia, Charlottesville.*
3. Electrophoretic studies of the soluble proteins of rabbit liver. *S. Sorof* and P. P. Cohen. Lab. of Physiological Chemistry, Univ. of Wisconsin, Madison.*
4. Release of various proteins from incubated surviving liver slices. *Rita L. Pressotti* and Ralph W. Brauer. Dept. of Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans (Pharmacol.).*
5. Studies on the origin of serum proteins. *Sidney Roberts* and Abraham White. Dept. of Physiological Chemistry, School of Medicine, Univ. of California at Los Angeles.*
6. Liver regeneration in the adrenalectomized rat. *Charles E. Friedgood*, Harry M. Vars, and Joan W. Zerbe*. Harrison Dept. of Surgical Research, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
7. Hormonal influences on cytochrome C metabolism and liver regeneration. *David L. Drabkin. Dept. of Physiological Chemistry, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*
8. Arginase, rhodanese and ATPase activity of regenerating livers in protein-depleted rats. *Otto Rosenthal, Charles S. Rogers* and Colin C. Ferguson*. Harrison Dept. of Surgical Research, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
9. Effect of dietary pteroylglutamic acid on liver D-amino acid oxidase of young rats. *Barbara Kelley (introduced by Paul L. Day). Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock.*
10. Preliminary communication on the effect of growth hormone on tissue enzymes. *James C. Mathies (introduced by Oliver H. Gaebler). Edsel B. Ford Inst. for Medical Research, Henry Ford Hospital, Detroit.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Carbohydrate Metabolism I

MURAL ROOM

1. Effects of indophenols on glycolysis. *James A. Freek (introduced by C. L. Gemmill). Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville (Pharmacol.).*
2. Effects of naphthoquinones and related compounds on glycolysis. *Chalmers L. Gemmill. Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville (Pharmacol.).*
3. A polarographic study of certain quinones. *James G. Hilton (introduced by C. L. Gemmill). Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville (Pharmacol.).*
4. Glycolysis and the Pasteur effect on the subcellular level. *Silvio Fiala (introduced by Dean Burk). National Cancer Inst., National Insts. of Health, Bethesda, Md.*
5. Anaerobic and aerobic glycolysis of parasitic tissue helminths. *Ernest Bueding. Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland.*
6. Effects of the posterior pituitary hormones on carbohydrate metabolism. *Alexander MacL. Fraser. Dept. of Pharmacology, McGill Univ., Montreal, Canada (Pharmacol.).*
7. Estimation of glycogen in liver and muscle. *Sam Seifter and Seymour Dayton*. Dept. of Biochemistry, Long Island College of Medicine, Brooklyn.*
8. Glycogen content of the liver of fasted alloxan diabetic rats. *Yoshikazu Morita* and James M. Orten. Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Symposium on Amino Acid Metabolism

FOUNTAIN BALLROOM

Joseph S. Fruton, *Chairman*

1. Proteolytic enzymes. *Emil L. Smith. Univ. of Utah, Salt Lake City.*
2. Incorporation of amino acids into proteins. *Henry Borsook. California Inst. of Technology, Pasadena.*
3. Mechanism of peptide bond formation. *Fritz Lipmann. Massachusetts General Hospital, Boston.*
4. Transamination and decarboxylation. *I. C. Gunsalus. Indiana Univ., Bloomington.*
5. Mechanism of urea synthesis. *Sarah Ratner. New York Univ.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Steroid Chemistry

LODGE ROOM E

1. Chromatography of urinary steroids and products of acid hydrolysis of pure steroids. *R. M. Archibald. Hospital of Rockefeller Inst. for Medical Research, New York City.*
2. A 16-oxygenated steroid from adrenocortical tumor urine. *H. Hirschmann and Frieda B. Hirschmann*. Dept. of Medicine, Western Reserve Univ.; Lakeside Hospital, Cleveland.*
3. Hydrolysis of conjugated 17-ketosteroids in urine concentrates by acetate buffers. *Joel Bitman* and Saul L. Cohen. Dept. of Physiological Chemistry, Univ. of Minnesota Medical School, Minneapolis.*
4. Effects of glucose, bile salts, and protein on recovery of neutral 17-ketosteroids. *William T. Beher* and P. H. Gaebler. Edsel B. Ford Inst. for Medical Research, Henry Ford Hospital, Detroit.*
5. Preparation of deuterated steroids. *David K. Fukushima* and Seymour Lieberman. Dept. of Steroid Biochemistry, Sloan-Kettering Inst. for Cancer Research, New York City.*
6. Application of micromethods to the study of steroid metabolism. *J. D. Hardy*, Hildegard Wilson* and Konrad Dobriner. Sloan-Kettering Inst. for Cancer Research; Dept. of Physiology, Cornell Univ. Medical College, New York City.*
7. Concerning groups involved in the Zimmerman and Kober reactions. *H. W. Marlow (introduced by H. C. Tidwell). Dept. of Biochemistry, Southwestern Medical College, Dallas.*
8. Inactivation of stilbestrol in alkali. *Raymond Vanderlinde* and W. W. Westerfeld. Dept. of Biochemistry, Syracuse Univ. College of Medicine, N. Y.*
9. Estimation of ergosterol with activated glycerol dichlorohydrin. *Romola Ettinger* and Albert E. Sobel. Polytechnic Inst.; Jewish Hospital, Brooklyn.*
10. Study of the carbohydrate-regulating hormone of hog adrenal extract. *William J. Haines*, Richard H. Johnson*, Mari P. Brunner*, Marvin E. Pabst* and Marvin H. Kuizenga. Research Labs., Upjohn Co., Kalamazoo, Mich.*
11. Chromatographic separation of bile acids. I. Separation of mixtures of cholic and desoxycholic acids. *Arthur F. Johnson (introduced by Arthur H. Smith). Dept. of Surgery, College of Medicine, Wayne Univ., Detroit.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Mineral Metabolism

ROOM 5

1. Relation of the electrolyte compositions of serum and skeletal muscle. *Edward Muntwyler, Grace E. Griffin*, George S. Samuelsen* and Lois E. Griffith*. Dept. of Biochemistry, Long Island College of Medicine, Brooklyn.*
2. Water and electrolyte losses from the skin of infants. *Edward L. Pratt*, Robert E. Cooke* and Daniel C. Darrow. Dept. of Pediatrics, Yale Univ. School of Medicine, New Haven, Conn.*
3. Chloride diarrhea and systemic alkalosis in potassium deficiency. *Lytt I. Gardner, Elsie A. MacLachlan, Mary L. Terry, Janet W. McArthur and Allan M. Butler (introduced by Ralph W. McKee). Children's Medical Service, Massachusetts General Hospital; Dept. of Pediatrics, Harvard Medical School, Boston.*
4. Biochemical and clinical effects of cationic exchange resin. *Theodore S. Cobbey, Jr.*, Robert H. Williams, Beverly T. Towery* and and Norma MacRae*. Dept. of Medicine, Univ. of Washington, Seattle (Pathol.).*
5. Distribution of radioisotopes of carbon and calcium administered to rats by continuous intraperitoneal infusion. *S. H. Zbarsky*, Roger Lienke* and W. D. Armstrong. Dept. of Physiological Chemistry, Univ. of Minnesota Medical School, Minneapolis.*
6. Polarographic micromethod for the determination of chloride in whole blood, serum and plasma. *Walter J. Zimmerman and William M. Layton, Jr. (introduced by H. Jensen). Medical Dept. Field Research Lab., Fort Knox, Ky.*
7. Principles of design and operation of internal standard flame photometers for sodium and potassium determination. *Robert L. Bowman and Robert W. Berliner (introduced by David P. Earle, Jr.). New York Univ. Research Service, Goldwater Memorial Hospital, New York (Physiol.).*
8. Variability in the Beckman spectrophotometer. *W. O. Caster* and Olaf Mickelsen. Nutrition Section, U. S. Public Health Service, Bethesda, Md.*
9. Thiocyanation of tyrosine. *Marie Easterwood* and John L. Wood. Dept. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis.*
10. Competitive antagonists of thyroxine and structurally related compounds. *Earl Frie-*

den and Richard J. Winzler. Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles.*

11. Behavior of the thyroid toward elements of the seventh periodic group. II. Rhenium. *Emil J. Baumann, N. Zizmer* and S. M. Seidlin*. Lab. Div. and Lab. of Medical Physics, Montefiore Hospital, New York City.*
12. Micro permanganate acid ashing method for determination of serum iodine. *Evelyn B. Man and Dorothy A. Siegfried*. Yale Univ. School of Medicine, New Haven, Conn.*

THURSDAY, APRIL 21, 9:00 A.M.

Tricarboxylic Acid Cycle

COMMANDERY ASYLUM

1. Oxalacetate decarboxylase of *azotobacter vinlandii*. *G. W. E. Plaut*, and Henry A. Lardy. Dept. of Biochemistry, Univ. of Wisconsin, Madison.*
2. Enzymatic synthesis of L-malic acid by CO_2 fixation. *Severo Ochoa, J. B. Veiga Salles*, and Priscilla J. Ortiz*. Dept. of Pharmacology, New York Univ. College of Medicine.*
3. Adaptive enzymatic conversion of L-malate to lactate and CO_2. *Seymour Korkes*, Alice Del Campillo*, and Severo Ochoa. Dept. of Pharmacology, New York Univ. College of Medicine.*
4. Conversion of isotopic pyruvate to oxalacetate by pigeon liver. *M. F. Utter with the technical assistance of Mary T. Chenoweth*. Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland.*
5. Studies on the mechanism of the biosynthesis of propionic acid. *D. S. Anthony, S. F. Carson, M. Kuna, and E. F. Phares (introduced by Alexander Hollaender). Biology Div., Oak Ridge National Lab., Oak Ridge, Tenn. (Physiol.).*
6. D-Aspartic oxidase of rabbit kidney and liver. *J. L. Still, and M. V. Buell (introduced by D. E. Green). Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*
7. Oxidation of higher fatty acids by isolated rat liver mitochondria. *Eugene P. Kennedy* and Albert L. Lehninger. Depts. of Biochemistry and Surgery, Univ. of Chicago.*
8. Action of streptomycin in tissue homogenates. *W. W. Umbreit and N. E. Tonhazy*. Merck Inst., Rahway, N. J.*
9. Antimycin antibiotics. *Kamaluddin Ahmad*, F. Merlin Bumpus*, B. R. Dunshee*, and F. M. Strong. Dept of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.*
10. Progesterone and testosterone reversal of diethylstilbestrol inhibition of succinoxidase of tumor mitochondrial elements. *Kent Wight* and Dean Burk. National Cancer Inst., National Inst. of Health, Bethesda, Md.*
11. Determination of fumaric acid in animal tissues by partition chromatography. *Lawrence M. Marshall*, James M. Orten, and Arthur H. Smith. Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit.*

THURSDAY, APRIL 21, 9:00 A.M.

Sulfur-containing Amino Acids and Transmethylation

LODGE ROOM E

1. Studies of the cystine and methionine content of portal blood plasma. *James D. Solomon* and Harold D. West. Dept. of Biochemistry, Meharry Medical College, Nashville.*
2. Effect of aromatic hydrocarbons on the metabolism of exogenous sulfur amino acids. *Helmut R. Gutmann* and John L. Wood. Dept. of Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis.*
3. Lipotropic action of threonine. *S. A. Singal, V. P. Sydenstricker* and Julia M. Littlejohn*. Univ. of Georgia School of Medicine, Augusta.*
4. Influence of lipotropic compounds on the lipid and protein fractions of blood plasma from children with nephrotic syndrome. *Chi Che Wang, H. H. Boyle and S. Freeman. Children's Memorial Hospital and Northwestern Univ. Medical School, Chicago.*
5. Effect of biotin and choline intakes on liver storage of ingested cholesterol. *Ruth Okey, Eleanor Risser Vernon*, S. Lepkovsky and R. Pencharz*. Dept. of Home Economics and the Div. of Poultry Research, Univ. of California, Berkeley.*
6. Effects of feeding an ethanolamine analogue (diethanolamine) on the formation of liver phospholipides. *Camillo Artom, W. E. Cornatzer* and Marietta Crowder*. Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.*
7. Effect of ethionine on fat metabolism and on protein formation. *E. Farber*, M. Simpson* and H. Tarver. Div. of Biochemistry, Univ. of California Medical School, Berkeley.*
8. Conversion of S^{35}-homolanthionine to S^{35}-cystine in the rat. *Jakob A. Stekol and Kathryn Weiss*. Lankenau Hospital Research Inst. and Inst. for Cancer Research, Philadelphia.*
9. Formation of formaldehyde in the biological oxidation of the methyl group of sarcosine.

Cosmo G. Mackenzie and Vincent duVigneaud. Dept. of Biochemistry, Cornell Univ. Medical College, New York City.

10. Non-lability of choline methyl and the role of choline oxidase in transmethylation. *Jacob W. Dubnoff. California Inst. of Technology, Pasadena.*
11. Cystathionase. *Francis Binkley. Lab. for the Study of Hereditary and Metabolic Disorders, Univ. of Utah School of Medicine, Salt Lake City.*

THURSDAY, APRIL 21, 9:00 A.M.

Phosphatases and Other Esterases

ROOM 5

1. Phosphatase activity in homogenates of liver and other organs of the rat. *Alex B. Novikoff (introduced by Harold B. Pierce). Dept. of Pathology, Univ. of Vermont College of Medicine, Burlington.*
2. Relationship of cell surface phosphatases to sugar metabolism in yeast. *Aser Rothstein and Rebecca Meier (introduced by H. C. Hodge). Atomic Energy Project of Univ. of Rochester, N. Y.*
3. Histochemical demonstration of three groups of alkaline (pH 9.2) phosphatases using various substrates and inhibitors. *William Newman*, Irwin Feigin*, Abner Wolf* and Elvin A. Kabat. Veterans Hospital, Kingsbridge; Depts. of Pathology and Neurology, Columbia Univ. and the Neurological Inst., New York City.*
4. Influence of magnesium and cobalt on the inhibition of phosphatases by α-amino acids. *Oscar Bodansky. Memorial Hospital for the Treatment of Cancer and Allied Diseases and the Sloan-Kettering Inst. for Cancer Research, New York City.*
5. Inhibitory action of some antioxidants on rat kidney phosphatase. *Carter D. Johnston (introduced by Edwin P. Laug). Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
6. Amylase and lipase in the intestinal lymph of the rat. *E. V. Flock and J. L. Bollman. Mayo Foundation, Rochester, Minn.*
7. Hydrolysis of esters by rabbit polymorphonuclear leucocytes. *R. J. Rossiter and Esther Wong*. Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*
8. Non-choline esters as substrates of e-choinesterase ("true" cholinesterase). *E. Albert Zeller, Gerard A. Fleisher and Robert A. McNaughton (introduced by Marschelle H. Power). Div. of Biochemistry, Mayo Foundation, Rochester, Minn.*
9. Study of the cholesterol esterase of pancreatin. *C. R. Treadwell, R. S. Yamamoto* and Norman P. Goldstein*. Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington.*
10. Studies on the phenolsulfatase of animal tissues. *Lynn D. Abbott, Jr. and Mary K. East*. Dept. of Biochemistry, Medical College of Virginia, Richmond.*

THURSDAY, APRIL 21, 1:30 P.M.

Amino Acid Metabolism II

COMMANDERY ASYLUM

1. A study of tryptophan metabolism using isotope tracer technique. *R. W. Schayer*, G. L. Foster and David Shemin. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.*
2. Abnormal excretion of tryptophan-like substances in tuberculosis. *Anthony A. Albanese, L. Emmett Holt, Jr., Marilyn Lein*, Emilie M. Smetak* and Betty Vestal. Dept. of Pediatrics, New York Univ. College of Medicine.*
3. Enzymes of liver oxidizing tryptophan to a kynurenine-like compound. *W. E. Knox, Kathleen Mero*, W. I. Grossman* and V. H. Auerbach*. Rheumatic Fever Research Inst., Northwestern Medical School, Chicago.*
4. Mechanism of histidine synthesis in lactic acid bacteria. *Harry P. Broquist* and Esmond E. Snell. Dept. of Biochemistry, Univ. of Wisconsin, Madison.*
5. Photoelectric estimation of L-carnosine and L-histidine. *G. A. Emerson and Paul L. Ewing. Dept of Pharmacology, Univ. of Texas Medical Branch, Galveston (Pharmacol.).*
6. D-serine inhibition of *E. coli* and its reversal by other acids and by mutation. *Werner K. Maas* and Bernard D. Davis. U. S. Public Health Service, Tuberculosis Research Lab., Cornell Univ. Medical College, New York; Naval Medical Research Inst., Bethesda, Md.*
7. Quantitation of the growth-retarding and anemia-producing effects of hexahomoserine. *E. T. Mertz, R. H. Waltz, Jr., D. C. Shelton, L. P. Doyle and A. L. Delez (introduced by F. W. Quackenbush). Depts. of Agricultural Chemistry and Veterinary Science, Purdue Univ., Lafayette, Ind.*
8. Synthesis and microbiological testing of some α-aminosulfonic acids. *Julius Mendel and Donald W. Visser (introduced by Harry J. Deuel, Jr.). Dept. of Biochemistry and Nutri-*

tion, Univ. of Southern California School of Medicine, Los Angeles.

9. Studies on transaminase in germinating seeds and its relation to protein synthesis. *Betsy Patterson Smith* and Harold H. Williams. Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.*
10. Transaminase activity in the skeletal muscles of normal and vitamin E-deficient animals. *Mary Alice Barber*, Daniel H. Basinski* and H. A. Mattill. Dept. of Biochemistry, State Univ. of Iowa, Iowa City.*
11. Analysis of certain components of skeletal muscle during vitamin E deficiency. *Charlotte E. Roderuck (introduced by H. A. Mattill). Dept. of Biochemistry, State Univ. of Iowa, Iowa City.*
12. An evaluation of some lactic acid producing bacteria in the microbiological assay of amino acids. *Myron Brin* and Harold H. Williams. Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.*

THURSDAY, APRIL 21, 4:30 P.M.

Biochemistry Business Meeting

COMMANDERY ASYLUM

THURSDAY, APRIL 21, 1:30 P.M.

Purine Metabolism

LODGE ROOM E

1. Determination of uric acid in whole blood and plasma. *Arnold H. Schein and Emily F. Rice (introduced by Harold B. Pierce). Dept. of Biochemistry, College of Medicine, Univ. of Vermont, Burlington.*
2. Interference of gentisic acid in determination of urinary uric acid after administration of salicylates. *T. F. Yu* and Alexander B. Gutman. Research Service, First (Columbia Univ.) Div., Goldwater Memorial Hospital; Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.*
3. Effect of certain mitotic poisons on uric acid and allantoin excretion in mice. *Allan D. Bass and Elizabeth F. Place. Dept. of Pharmacology, College of Medicine, Syracuse, (Pharmacol.).*
4. Effect of uric acid on the preoxidative detoxification of diphtheria toxin. *Kuell Agner (introduced by J. W. Williams). Dept. of Chemistry, Univ. of Wisconsin, Madison.*
5. Fate of ingested uric acid in man. *Aaron Bendich*, William D. Geren*, Oscar Bodansky and George Boswath Brown. Sloan-Kettering Inst. for Cancer Research, New York.*
6. Study in human uric acid metabolism. *Jean D. Benedict*, Peter H. Forsham* and DeWitt Stetten, Jr. Dept of Biological Chemistry, Harvard Medical School; Dept. of Medicine, Peter Bent Brigham Hospital; Div. of Nutrition and Physiology, Public Health Research Inst. of The City of New York, Inc.*
7. Liver respiration as related to its xanthine oxidase activity. *Dan A. Richert* and W. W. Westerfeld. Dept. of Biochemistry, Syracuse Univ. College of Medicine.*
8. Enzymatic degradation of thymidine. *L. A. Manson* and J. O. Lampen. Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis.*
9. Determination of pyrimidines by the isotope derivative method. *Robert C. Warner. Dept. of Chemistry, New York Univ. College of Medicine.*
10. Isotopic studies of the biosynthesis of nucleic acid components. II. Allantoin. *Jerome D. Valentine*, Samuel Gurin and D. Wright Wilson. Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
11. Studies on hypoxanthine synthesis in pigeon liver with labeled formic acid. *G. Robert Greenberg (introduced by Harland G. Wood). Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland.*
12. Isotopic studies of the biosynthesis of nucleic acid components. I. Purines and pyrimidines. *Milton R. Heinrich* and D. Wright Wilson. Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
13. Incorporation of C^{14}-formic acid into nucleic acid fractions of rat and pigeon. *Walton H. Marsh (introduced by Harland G. Wood). Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland.*

THURSDAY, APRIL 21, 4:30 P.M.

Biochemistry Business Meeting

COMMANDERY ASYLUM

THURSDAY, APRIL 21, 1:30 P.M.

Hyaluronidase, Glucuronidase and Lysozyme

ROOM 5

1. Preparation of hyaluronate from some mammalian sources. *Sibylle Tolksdorf*, James W. Cassidy*, Carol Bonsal* and D. Roy McCul-*

lagh. Dept. of Biochemistry, Schering Corporation, Bloomfield, N. J.

2. Stability and stabilization of testicular hyaluronidase. *D. Roy McCullagh, Florence Valentine* and Sibylle Tolksdorf*. Dept. of Biochemistry, Schering Corp., Bloomfield, N. J.*
3. *In vitro* studies on the inhibition of hyaluronidase activity. *Harvey E. Alburn, Robert W. Whitley and Louise C. Hall (introduced by Sam Seifter). Wyeth Inst. of Applied Biochemistry, Philadelphia.*
4. Inhibition of hyaluronidase by blood serum bile constituents. *David Glick, Lee W. Wattenberg* and Erick Y. Hakanson*, Dept. of Physiological Chemistry, Univ. of Minnesota Medical School, Minneapolis.*
5. Effect of a series of flavanoids on hyaluronidase activity. *Gertrude Rodney, L. M. Wheeler and Ann Swanson (introduced by J. J. Pfiffner). Research Labs., Parke Davis & Co., Detroit.*
6. *In vitro* action of gentisic acid on hyaluronidase. *Saul Roseman*, Faith Pearson* and Albert Dorfman. Dept. of Pediatrics, Univ. of Chicago.*
7. Influence of estrogenic hormones on B-glucuronidase in the human. *William H. Fishman and Lester D. Odell*. Depts of Surgery and Biochemistry, Obstetrics and Gynecology, Univ. of Chicago.*
8. Studies on bacterial glucuronidase. *Henry J. Buehler*, Philip A. Katzman and Edward A. Doisy. Lab. of Biological Chemistry, Saint Louis Univ. School of Medicine.*
9. Studies on lyxozyme. *Charles M. Proctor and Sherman R. Dickman (introduced by Emil L. Smith). Dept. of Biological Chemistry, Univ. of Utah Medical School, Salt Lake City.*
10. Mechanism of reversible inactivation of lysozyme by iodine and sulfite. *H. Fraenkel-Conrat. Western Regional Research Lab. 1 Albany, Calif.*

THURSDAY, APRIL 21, 4:30 P.M.

Biochemistry Business Meeting

COMMANDERY ASYLUM

FRIDAY, APRIL 22, 9:00 A.M.

Carbohydrate Metabolism II

COMMANDERY ASYLUM

1. Preliminary studies on the metabolism of radioactive sucrose in the rat. *Alvin R. Lamb and George O. Burr. Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii.*
2. Direct utilization of sucrose by *Leuconostoc*. *Warner W. Carlson and Virginia Whiteside Carlson (introduced by Emmett B. Carmichael). Biochemistry Dept., Medical College of Albama, Birmingham.*
3. Studies in the metabolism of D-arabinose. *Joseph H. Roe, Eugene W. Rice* and Benjamin W. Smith*. Dept. of Biochemistry, School of Medicine, George Washington Univ., Washington.*
4. Degradation of polysaccharides by muscle phosphorylase. *Shlomo Hestrin (introduced by C. F. Cori). Dept. of Biological Chemistry, School of Medicine, Washington Univ., St. Louis.*
5. Rat liver fructokinase. *Carl S. Vestling, Ursula Irish* Augusta K. Hirsch* and Norman H. Grant*, Div. of Biochemistry, W. A. Noyes Lab. of Chemistry, Univ. of Illinois, Urbana.*
6. Mechanism of action of phosphoglucomutase. *Venkataraman Jagannathan* and James Murray Luck. Dept. of Chemistry, Stanford Univ., Stanford, Calif.*
7. Activation of phosphoglucomutase. *E. Sutherland*, T. Posternak* and C. F. Cori. Biological Chemistry Dept., Washington Univ. School of Medicine, St. Louis.*
8. Phosphomannose isomerase. *Milton W. Slein (introduced by Gerty T. Cori). Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis.*
9. Specificity of liver glucose-6-phosphatase. *Marjorie A. Swanson (introduced by Cammillo Artom). Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.*

FRIDAY, APRIL 22, 9:00 A.M.

Steroid and Fat Metabolism

LODGE ROOM E

1. Metabolism of 16-ketoestrone and 16-keto-α-estradiol in man. *Benjamin F. Stimmel, Arthur Grollman and Max N. Huffman. Rees-Stealy Medical Research Fund, Ltd., San Diego; Depts. of Pharmacology and Experimental Medicine and Biochemistry, Southwestern Medical College, Dallas.*
2. Metabolism of dehydroisoandrosterone in a woman before and after removal of an adrenocortical tumor. *Harold L. Mason, John J. Schneider* and Edwin J. Kepler*. Div. of Biochemistry and Div. of Medicine, Mayo Clinic, Rochester, Minn.*

3. Metabolism of testosterone and androstanediol-3α, 17α by liver and kidney homogenates. *Charles D. Kochakian, N. Parente* and H. V. Aposhian*. Dept. of Physiology and Vital Economics, School of Medicine and Dentistry, Univ. of Rochester, N. Y.*
4. *In vitro* metabolism of testosterone and its metabolites by kidney tissue. *Charles Donald West* and Leo T. Samuels. Dept. of Biochemistry, Univ. of Utah, Salt Lake City.*
5. Metabolism of α-estradiol *in vitro*. *W. H. Pearlman and R. H. DeMeio*. Dept. of Biochemistry, Jefferson Medical College, Philadelphia.*
6. Effect of bixin upon the rate of autoxidation of methyl linoleate. *H. O. Kunkel and Walter L. Nelson (introduced by James B. Sumner). Dept. of Biochemistry and Nutrition, Cornell Univ., Ithaca, N. Y.*
7. Serum lipides of Eskimos. Effect of a high fat diet (Pemmican) and of fasting. *R. G. Sinclair, G. M. Brown* and L. B. Cronk*. Depts. of Biochemistry and Medicine, Queen's Univ., Kingston, Ontario, Canada.*
8. Effect of physical stress on the liver fat content of the fasted mouse. *Louis Levin. Dept. of Anatomy, College of Physicians and Surgeons, Columbia Univ., New York City.*
9. Lecithinase activity of epiphyseal cartilage of of the foetal pig. *Milton D. Levine and Richard H. Follis, Jr. (introduced by John Eager Howard). Depts. of Medicine and Pathology, Johns Hopkins Univ., Baltimore (Physiol.).*

FRIDAY, APRIL 22, 9:00 A.M.

Proteolytic Enzymes

ROOM 5

1. Isolation of a crystalline heat stable trypsin inhibitor from lima beans. *Henry Tauber. V. D. Research Lab., U. S. Public Health Service, U. S. Marine Hospital, Staten Island, N. Y.*
2. Some properties of chymotrypsinogen B and chymotrypsin B. *Virginia Kubacki*, Kenneth D. Brown* and M. Laskowski. Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee.*
3. Mode of inhibition of chymotrypsin by DFP. II. Elimination of fluorine and introduction of isopropyl. *Eugene F. Jansen, Fellows Nutting*, Rosie Jang* and A. K. Balls. Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Albany, Calif.*
4. Effect of dihydroxyphenylalanine on enzymatic digestion. *M. X. Sullivan and S. Stephen Schiaffino*. Chemo-Medical Research Inst., Georgetown Univ., Washington, D. C.*
5. Depolymerase activities of calf spleen and thymus cathepsin preparations. *Mary E. Maver and Antoinette Greco*. National Cancer Inst., Bethesda, Md.*
6. Effect of pH and temperature on carboxypeptidase activity. *Hans Neurath and Elaine Elkins-Kaufman*. Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.*
7. Enzymatic hydrolysis of carnosine and related peptides. *H. Theo Hanson* and Emil L. Smith. Lab. for the Study of Hereditary and Metabolic Disorders and Depts. of Biochemistry and Medicine, Univ. of Utah School of Medicine, Salt Lake City.*
8. Partial separation of D- and L-peptidases and analogous dehydropeptidase I. *Vincent E. Price*, Alton Meister* and Jesse P. Greenstein. National Cancer Inst., National Inst. of Health, Bethesda, Md.*
9. Observations on the equilibrium between glycine and glycylglycine in the presence of liver peptidase. *Ivan D. Frantz, Jr., Robert B. Loftfield and Ann S. Werner (introduced by Fritz Lipmann). Medical Labs. of Collis P. Huntington Memorial Hospital of Harvard Univ. at the Massachusetts General Hospital, Boston.*
10. Some factors which influence the synthesis of peptide bonds as catalyzed by papain. *Cornelius W. Pettinga* and Sidney W. Fox. Chemical Lab., Iowa State College, Ames.*
11. Synthesis of some benzoylamino acid anilides as catalyzed by papain. *Sidney W. Fox, Frederick N. Minard*, Harry Wax*, Cornelius W. Pettinga* and Jacquetta Strifert*. Chemical Lab. Iowa State College, Ames.*

FRIDAY, APRIL 22, 1:30 P.M.

Amino Acid Metabolism III

COMMANDERY ASYLUM

1. An enzymatic reaction involving amino acids and adenosine triphosphate. *John M. Reiner. Dept. of Physiology, Tufts College Medical School, Boston (Physiol.).*
2. Further studies on enzymatic benzoylations. *R. W. McGilvery* and P. P. Cohen. Lab. of Physiological Chemistry, Univ. of Wisconsin, Madison.*
3. Analysis of protein hydrolysates by group separation and paper chromatography of

isotopically labelled derivatives. *Albert S. Keston*, Sidney Udenfriend* and Milton Levy. Dept. of Chemistry, New York Univ. College of Medicine.*

4. Incorporation of radioactive amino acids into the protein of acellular liver homogenate fractions. *Elbert A. Peterson*, Theodore Winnick and David M. Greenberg. Div. of Biochemistry, Univ. of California Medical School, Berkeley.*
5. Concentration of amino acids by the rat diaphragm suspended in artificial media. *Halvor N. Christensen. Children's Hospital; Dept. of Biological Chemistry, Harvard Medical School, Boston.*
6. Effect of penicillin upon amino acid assimilation in staphylococci. *Rollin D. Hotchkiss. Rockefeller Inst. for Medical Research, New York City.*
7. Formation of serine from glycine in the intact rat. *Warwick Sakami (introduced by Harland G. Wood). Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland.*
8. Biological synthesis of serine from glycine. *Philip Siekevitz*, Theodore Winnick and David M. Greenberg. Div. of Biochemistry, Univ. of California Med. Sch., Berkeley.*
9. Biosynthesis of heme. *Norman S. Radin*, D. Rittenberg and David Shemin. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.*
10. Effect of x-ray radiation and starvation on hemoglobin synthesis in the rat. *K. Salomon, K. I. Altman, G. W. Casarett and T. R. Noonan (introduced by F. L. Haven). Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, N. Y.*
11. Metabolic conversion of the carbon chain of DL-lysine-ϵ-C^{14} to L-glutamic and L-aspartic acids in the dog. *Leon L. Miller and William F. Bale*. Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, N. Y.*

FRIDAY, APRIL 22, 1:30 P.M.

Nucleic Acids, Nucleotides and Coenzymes

LODGE ROOM E

1. Extraction and estimation of desoxypentose nucleic acid (DNA) and pentose nucleic acid (PNA) from plant tissues. *Maurice Ogur and Gloria Rosen (introduced by H. G. Albaum). Botanical Labs., Univ. of Pennsylvania, Philadelphia.*
2. Intracellular heterogeneity of liver ribonucleic acid as evidenced by P^{32} uptake. *Cyrus P. Barnum and Robert A. Huseby*. Depts. of Physiological Chemistry and Physiology, Univ. of Minnesota Medical School, Minneapolis.*
3. Chromatographic separation of ribonucleotides and its application to the study of ribonucleic acid structure. *Ernst Vischer*, Boris Magasanik* and Erwin Chargaff. Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.*
4. Separation of three naturally occurring adenine ribonucleotides by paper chromatography and ion-exchange. *C. E. Carter* and Waldo E. Cohn. Biology Div., Oak Ridge National Lab., Tenn.*
5. Isolation of adenosine triphosphate from plant tissue. *H. G. Albaum, M. Ogur* and A. Hirshfeld*. Depts. of Biology and Chemistry, Brooklyn College.*
6. Bound coenzymes of the cyclophorase complex of rabbit kidney and liver. *L. J. Teply (introduced by D. E. Green). Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*
7. Concentration of bound pantothenic acid. *J. B. Neilands*, Harvey Higgins* and F. M. Strong. Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.*
8. Studies of nuclei isolated from normal rat and rabbit liver by a modified Behrens technique. *Alexander L. Dounce, Garson H. Tishkoff*, Shirley R. Barnett* and Richard M. Freer*. Dept. of Pathology and Biochemistry, School of Medicine and Dentistry, Univ. of Rochester, N. Y.*
9. Phosphorus metabolism of molds. I. Uptake and transformation of orthophosphate by *Penicillium chrysogenum* Q176. *J. F. Snell* and W. H. Peterson. Univ. of Wisconsin, Madison.*
10. Effect of Theiler's GD VII virus on P^{32} uptake of one-day-old mouse brain cultures. *Max E. Rafelson, Jr.*, Harold E. Pearson* and Richard J. Winzler. Depts. of Biochemistry and Bacteriology, Univ. of Southern California School of Medicine; Lab. Div. of Los Angeles County Hospital.*
11. Fate of the infecting virus particle. *Lloyd M. Kozloff* and Frank W. Putnam. Dept. of Biochemistry, Univ. of Chicago.*

FRIDAY, APRIL 22, 1:30 P.M.

Blood

ROOM 5

1. Comparison of the electrophoretic components of human serum at pH 4.5 and 8.4. *John W. Mehl, Florence Golden* and Jane Humphrey*. Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine; Los Angeles County General Hospital.*
2. Electrophoretic studies of the proteins of plasma and ascitic fluid in cirrhosis. *W. D. Paul*, J. D. Gordon*, Robert L. Dryer* and Jospeh I. Routh. Depts. of Medicine and Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City.*
3. Electrophoretic and chemical analyses of plasma and its fractions in health and disease. *H. Rowland Pearsall* and Alfred Chanutin. Biochemical Lab., Univ. of Virginia, Charlottesville.*
4. Electrophoretic patterns of plasma proteins during pregnancy and following delivery. *Margaret N. Coryell*, Eliot F. Beach and Abner Robinson*. Research Lab., Children's Fund of Michigan, Detroit.*
5. Protein changes in the developing chick embryo. *Margaret E. Marshall* and H. F. Deutsch. Dept. of Physiological Chemistry, Univ. of Wisconsin, Madison.*
6. Determination of serum protein. Studies of agents for separation of albumin and globulin. *John G. Reinhold, L. W. Bluemle, Jr.*, Francisco Campoy*, Logan Gilman*, Edward F. Golud*, Vernon L. Martens* and Richard Shuman*. Pepper Lab. of Clinical Medicine, Nutritional Service, Hospital of Univ. of Pennsylvania, Philadelphia.*
7. Determination of gamma globulin in blood serum. *Joseph M. Looney and Mary O. Amdur*. Research Lab., Veterans Administration Hospital, West Roxbury, Mass.*
8. Interaction between hematin and serum albumin. *Morris Rosenfeld and Douglas M. Surgenor*. Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins School of Medicine, Baltimore; Dept. of Physical Chemistry, Harvard Medical School, Boston (Pharmacol.).*
9. Molecular weight of human fetal hemoglobin. *John Fuller Taylor and Richard L. Swarm*. Dept. of Biological Chemistry, Washington Univ. School of Medicine, St. Louis.*
10. Difference in electrophoretic behavior of sickle cell hemoglobin and normal human hemoglobin. *Harvey A. Itano and Linus Pauling (introduced by Carl G. Niemann). Gates and Grellin Labs. of Chemistry, California Inst. of Technology, Pasadena.*
11. Factors affecting the diazotization of bilirubin. *Betty King Proffitt* and Dempsie B. Morrison. Dept. of Chemistry, Univ. of Tennessee, Memphis.*
12. Determination of hemochromogen in serum. *William M. McCord (introduced by J. Roy Doty). Dept. of Chemistry, Medical College of South Carolina, Charleston.*

Papers Read by Title

1. Effect of the administration parenteral and oral casein hydrolysates to depleted dogs. *Carl Alper*, Bacon F. Chow and Shirley DeBiase*. Nutrition Dept., Div. of Development, and Div. of Protein Chemistry, Squibb Inst. for Medical Research, New Brunswick, N.J.*
2. Determination of dimethylethanolamine in biological materials. *Camillo Artom and Marietta Crowder*. Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N.C.*
3. Partial purification and properties of hydantoinase. *Frederick Bernheim, Mary L. C. Bernheim* and G. S. Eadie*. Depts. of Physiology, Pharmacology and Biochemistry, Duke Univ. Medical School, Durham, N. C.*
4. Action of ribonuclease on ribonucleic acid. *James E. Bacher* and Frank Worthington Allen. Div. of Biochemistry, Univ. of California Medical School, Berkeley.*
5. Tuberculostatic properties of autoclaved human plasma. *Donald E. Bowman. Depts. of Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis.*
6. Changes in sodium sulfate solutions in the upper and lower intestines of dogs. *Gladys R. Bucher*, Carl E. Anderson* and C. S. Robinson. Dept. of Biochemistry, Vanderbilt Univ., Nashville.*
7. Toxicity studies with demerol hydrochloride (isonipecaine) on adult male guinea pigs. *Emmett B. Carmichael, Walter H. Johnson* and Frank A. Kay*. Biochemistry Dept., Medical College of Alabama, Birmingham.*
8. Effect of oral ingestion of histidine on the glutamic acid blood level in rats. *H. R. Crookshank and Bernard F. Clowdus (introduced by Emmett B. Carmichael). Biochemistry Dept., Medical College of Alabama, Birmingham.*
9. Electrophoretic comparison of plasma and body fluid proteins in certain diseases. *Robert L. Dryer*, Jacqueline H. Budde*, W. D. Paul* and Joseph I. Routh. Depts. of Bio-*

chemistry and Medicine, College of Medicine, State Univ. of Iowa, Iowa City.

10. Lipids of the nervous system during *in vitro* degeneration. *A. C. Johnson*, A. R. McNabb* and R. J. Rossiter. Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*
11. Quantitative aspects of coprecipitation in the purification of substances isolated in tracer studies. *Albert S. Keston*, Sidney Udenfriend*, Nora Levitas* and Milton Levy. Dept. of Chemistry, New York Univ. College of Medicine.*
12. Penicillin-cytochrome complexes. *I. M. Klotz and W. W. Weber*. Dept. of Chemistry, Northwestern Univ., Evanston, Ill.*
13. Photoelectric colorimetry of pH. *Granvil C. Kyker. Dept. of Biological Chemistry and Nutrition, School of Medicine, Univ. of North Carolina, Chapel Hill.*
14. Relative size of adrenocorticotropically active peptide fragments. *Choh Hao Li. Inst. of Experimental Biology, Univ. of California, Berkeley.*
15. Manometric determination of methyl alcohol in blood. *M. F. Mason. Parkland Hospital and Southwestern Medical College, Dallas.*
16. Formazyl color reaction as a possible biochemical analytical tool. *Norman L. Matthews (introduced by Earl R. Norris). Dept. of Pharmacology, Univ. of Washington, Seattle; Emory Univ. School of Medicine, Atlanta; Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
17. Hepato-renal factors in circulatory homeostasis XXI. Quantitative immunochemistry of ferritin and apoferritin. *Abraham Mazur and Ephraim Shorr*. Dept. of Medicine, Cornell Univ. Medical College and The New York Hospital, New York City.*
18. Electrometric method for the determination of red blood cell and plasma cholinesterase activity. *Harry O. Michel (introduced by William H. Summerson). Biochemistry Section, Medical Div., Army Chemical Center, Md.*
19. Metabolic conversion of the carbon chain of DL-lysine- $-C^{14}$ to L-arginine in the dog. *Leon L. Miller. Dept. of Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
20. Enzymatic mechanism of arginine synthesis. *S. Ratner and Anne Pappas*. Dept. of Pharmacology, New York Univ., College of Medicine.*
21. Studies on prolamines treated with nitrogen trichloride. II. Acetylated zein. *L. Reiner, Fernanda Misani*, Philip Weiss*, M. G. Cordasco* and T. W. Fair*. Research Labs., Wallace & Tiernan Products, Inc., Bloomfield, N. J.*
22. Tissue collagen in experimental scurvy. *Wm. v. B. Robertson (introduced by H. B. Pierce). Div. of Experimental Medicine and Dept. of Biochemistry, College of Medicine, Univ. of Vermont, Burlington.*
23. Chemical changes in isotonic saline solutions placed in successively lower levels of human small intestine. *C. S. Robinson, Gladys R. Bucher* and D. Dziewiatkowski*. Dept. of Biochemistry, Vanderbilt Univ., Nashville.*
24. Effects of India ink injections in avitaminosis A. *Stelios C. Samaras*, Nicholas Dietz, Jr.* and Victor E. Levine. Dept. of Biological Chemistry and Nutrition, Creighton Univ. School of Medicine, Omaha.*
25. Optical and side-chain specificity of leucine amino-peptidase. *Emil L. Smith and W. J. Polglase*. Lab. for the Study of Hereditary and Metabolic Disorders, and Depts. of Biochemistry and Medicine, Univ. of Utah, School of Medicine, Salt Lake City.*
26. Combined ultraspectrographic and chemical studies in cerebrospinal fluids (CSFs) in normal and pathologic conditions. *M. Speigel-Adolf and H. T. Wycis*. Depts. of Colloid Chemistry and Neurosurgery, Temple Univ. Medical School and Hospital, Philadelphia.*
27. Relative importance of plasmatic and vascular factors of hemostasis in the hemorrhagic diathesis of liver dysfunction. *Mario Stefanini (introduced by A. J. Quick). Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee and Dept. of Internal Medicine, Univ. of Romas, Italy.*
28. L-Alanine oxidase of rabbit kidney and liver. *J. L. Still and M. V. Buell (introduced by D. E. Green). Inst. of Enzyme Research, Univ. of Wisconsin, Madison.*
29. Changes in the cholesterol concentration of rat liver homogenates during incubation. *Leon Swell* and C. R. Treadwell. Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington, D. C.*
30. Dietary effects on liver xanthine oxidase. *W. W. Westerfeld and Dan A. Richert*. Dept. of Biochemistry, Syracuse Univ. College of Medicine.*
31. Metabolism of tissue cultures of mouse-brain in relation to propagation of Theiler's GD VII virus. *Richard J. Winsler and Harold E. Pearson*. Depts. of Biochemistry and Bacteriology, Univ. of Southern California School of Medicine and the Lab. Div. of the Los Angeles County Hospital.*
32. Prolongation of pentobarbital sodium anes-

thesia by nitrates and nitrites. *Harold Wooster* and F. William Sunderman. Mellon Inst., Pittsburgh; Cleveland Clinic.*

33. Effect of antimalarial agents on adenosine triphosphatase of snake venoms. *E. Albert Zeller (introduced by Marschelle H. Power). Div. of Biochemistry, Mayo Foundation, Rochester, Minn.*

34. Effect of ultrasonic vibrations on the release and inactivation of paramecin. *L. P. Zill* and W. J. van Wagtendonk. Dept. of Zoology, Indiana Univ., Bloomington.*

THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Thirty-ninth Annual Meeting

An asterisk following an author's name denotes "by invitation"

TUESDAY, APRIL 19, 9:00 A.M.

Hypnotics and Analgesics

LODGE ROOM A

A. P. Richardson, *Chairman*

1. A modified ultraviolet spectrophotometric method for quantitative determination of barbiturates. *Thelma C. Gould, Elizabeth L. Mayo and Karl P. Bowman (introduced by Charles H. Hine). Divisions of Pharmacology and Experimental Therapeutics and of Psychiatry, Univ. of California Medical School, and Langley Porter Clinic, State Dept. of Mental Hygiene, San Francisco.*
2. Thiopental hypnosis in rabbits on experimental diets. *B. De Boer. Dept. Pharmacology, St. Louis Univ. School of Medicine.*
3. The liver as the major organ involved in the detoxication of thiopental by the dog. *A. R. Kelly* and F. E. Shideman. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
4. Some metabolic factors contributing to the course of pentothal anesthesia. *Claudia M. Ratliff,* A. H. Maloney, James R. Tureman* and Walter M. Booker. Dept. Pharmacology, School of Medicine, Howard Univ., Washington, D. C.*
5. Changes in blood volume during prolonged pentothal anesthesia. *James R. Tureman,* A. H. Maloney, Walter M. Booker and Claudia M. Ratliff.* Dept. Pharmacology, College of Medicine, Howard Univ., Washington, D. C.*
6. Fate of pentothal in man. *Lester C. Mark,* Philip A. Lief,* E. M. Papper,* E. A. Rovenstine, Eleonore Bernstein* and Bernard B. Brodie. Depts. Anesthesia and Biochemistry, New York Univ. College of Medicine, and New York Univ. Research Service, Goldwater Memorial Hospital, New York City.*
7. Effects of ions and enzyme inhibitors on breakdown of pentobarbital by rat liver slices. *J. B. Kahn, Jr. (introduced by J. M. Coon). Dept. Pharmacology, Univ. of Chicago, Ill.*
8. Pentobarbital tolerance in rabbits. *B. A. Westfall (introduced by J. K. W. Ferguson). Dept. Physiology and Pharmacology, Medical School, Univ. of Missouri, Columbia.*
9. Effect of pentobarbital on response of the cardiovascular system of dogs to epinephrine, acetylcholine and tilting. *James L. Morrison, Harry A. Walker and Arthur P. Richardson. Dept. Pharmacology, Emory Univ. School of Medicine, Emory Univ., Ga.*
10. A warm wire analgesimeter. *R. E. Lee,* H. L. Williams* and Carl C. Pfeiffer. Dept. Pharmacology, Univ. of Illinois College of Medicine, Chicago.*
11. Quality and potency determination of analgetics. *D. D. Bonnycastle and Johannes Ipsen, Jr.* Laboratory of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.*
12. The physiological disposition of aminopyrine in man. *Julius Axelrod* and Bernard B. Brodie. Dept. Biochemistry, New York Univ. College of Medicine, and New York Univ. Research Service, Goldwater Memorial Hospital and Laboratory of Industrial Hygiene, New York City.*
13. Respiration apparatus for rabbits and its use in the study of piperidine analgesics. *R. H. K. Foster and B. DeBoer. Dept. Pharmacology, St. Louis University School of Medicine.*

TUESDAY, APRIL 19, 9:00 A.M.

Autonomic Agents

ROOM 2

E. J. Fellows, *Chairman*

1. An attempt to systematize the actions of epinephrine substitutes on smooth muscle organs. *C. H. Thienes, Jack Lanley* and Walter Mattison*. School of Medicine, Univ. of Southern California, Los Angeles.*
2. Bronchodilator action of butanol derivatives. *A. M. Lands, O. H. Siegmund* and Estelle Ananenko,* Biology Division, Sterling-Winthrop Research Institute, Rensselaer, N. Y.*
3. Vasoconstrictor activity of epinephrine, ephedrine and a new sympathomimetic diamine 1-(m-hydroxyphenyl)-N^2-methylethylene diamine (NU-1683). *Howard Wayne,* Lewis Franklin* and Harold D. Green. Dept. Physiology and Pharmacology,*

Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.

4. Effects of epinephrine, ephedrine and 1-(m-hydroxyphenyl)-N²-methyethylene diamine (NU-1683) on the coronary blood flow. *Adam B. Denison, Jr.,* Ben J. Lawrence, Jr.* and Harold D. Green. Dept. Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.*
5. Adrenergic-like action of acetylcholine on the isolated rabbit heart. *H. D. Bruner and Kwang Soo Lee.* Dept. Pharmacology, Univ. of North Carolina, Chapel Hill.*
6. Observations on several cyclohexyl and phenyl alkylamines. *Edward Macko* and Edwin J. Fellows. Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia.*
7. Tachyphylaxis of sympathomimetic drugs. *Lloyd D. Seager and William Weiss.* Dept. Pharmacology and Anesthesiology, Woman's Medical College of Pennsylvania, Philadelphia.*
8. Differentiation of sympathetic from parasympathetic drugs by measurements of phase-boundary potentials. *T. C. Barnes and R. Beutner. Hahnemann Medical College and Hospital of Philadelphia.*
9. Studies on the metabolism of radioactive nicotine. *A. Ganz (introduced by F. E. Kelsey). Dept. Pharmacology, Univ. of Chicago.*
10. Studies on the pharmacology of N, 1-dimethyl hexylamine (Oenethyl). *J. H. Wills. Dept. Pharmacology, Univ. of Tennessee, Memphis (Physiol.).*
11. A new colorimetric reaction for the determination of epinephrine. *Samuel Natelson and Joseph B. Pincus (introduced by Albert E. Sobel). Depts. Chemistry and Pediatrics, Jewish Hospital of Brooklyn, N. Y. (Biochem.).*

TUESDAY, APRIL 19, 1:30 P.M.

Antibiotics

LODGE ROOM A

C. F. Schmidt, *Chairman*

1. Synergistic chemotherapeutic effect of penicillin and aurothioglucose (solganal). *Arnold Grandage, Leila Diamond, Marvin Rosenkrantz, Jeanne H. Chase, Richard Tislow and Erwin Schwenk (introduced by H. B. Haag). Biological Research Laboratories, Schering Corporation, Bloomfield, N. J.*
2. Potentiation by means of carbon dioxide of the action of penicillin against pneumococcus infection. *Richard W. Whitehead, J. Howard Williamson,* William E. Clapper,* Joseph N. Spencer* and William B. Draper. Depts. Pharmacology and Bacteriology, Univ. of Colorado Medical Center, Denver.*
3. Influence of roentgen rays on potency of penicillin. *Marcus Ostro* and David I. Macht. Depts. Pharmacology and Radiology, Sinai Hospital, Baltimore.*
4. Guanidine: the agent responsible for the convulsive response to intracisternally injected streptomycin. *S. Kuna,* F. Cuchie* and H. Molitor. Merck Institute for Therapeutic Research, Rahway, N. J.*
5. Renal clearance of streptomycin. *A. O. Edison,* V. C. Jelinek* and G. E. Boxer. Merck Institute for Therapeutic Research and the Research Laboratories of Merck & Co., Inc., Rahway, N. J.*
6. Binding of streptomycin to plasma proteins. *G. E. Boxer, V. C. Jelinek* and A. O. Edison.* Research Laboratories of Merck & Co., Inc., and the Merck Institute for Therapeutic Research, Rahway, N. J.*
7. Streptomycin content of brain and other tissues after parenteral injection. *J. E. Hawkins, Jr., G. E. Boxer and V. C. Jelinek.* Merck Institute for Therapeutic Research and the Research Laboratories of Merck & Co., Rahway, N. J.*
8. Aureomycin: protective effect in experimental Rocky Mountain spotted fever with particular reference to intermittent treatment. *Ludwik Anigstein, Dorothy M. Whitney and Joe Beninson (introduced by G. A. Emerson). Rickettsial Research Laboratory, Dept. Preventive Medicine and Public Health, Univ. of Texas Medical Branch, Galveston.*
9. Comparison of the actions of chloramphenicol (chloromycetin) and penicillin G against experimental relapsing fever. *Paul E Thompson and Mary C. Dunn (introduced by A. C. Bratton). Research Laboratories, Parke, Davis & Co., Detroit.*
10. Interrelationship between chloramphenicol (chloromycetin) and enzymatic systems. *Grant N. Smith and Cecilia Worrell (introduced by O. D. Bird). Research Laboratories, Parke, Davis & Co., Detroit (Biochem.)*
11. Chloramphenicol (chloromycetin), a new antibiotic; toxicity, absorption, excretion and pathology in animals. *O. M. Gruhzit, R. A. Fiskin,* T. F. Reutner* and Edith Martino.* Research Laboratories, Parke, Davis & Co., Detroit (Pathol.).*
12. Tissue distribution, excretion and metabolic fate of chloramphenicol (chloromycetin).

Anthony J. Glazko, Loretta M. Wolf and Wesley A. Dill.* Research Laboratories, Parke, Davis & Co., Detroit (Physiol.).*

13. Identification of chloramphenicol (chloromycetin) degradation products by paper chromatography. *Wesley A. Dill* and Anthony J. Glazko. Research Laboratories, Parke, Davis & Co., Detroit (Physiol.).*

TUESDAY, APRIL 19, 4:15 P.M.

Pharmacology Business Meeting

LODGE ROOM A

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

Program on page 418

TUESDAY, APRIL 19, 1:30 P.M.

Drug Toxicity

ROOM 2

O. G. Fitzhugh, *Chairman*

1. Loss of As from tissues by volatilization. *Randolph Clements,* J. Eugene Hill,* Hershel G. Tree,* P. L. Ewing and G. A. Emerson. Dept. Pharmacology, Univ. of Texas Medical Branch, Galveston.*
2. Arsenic content of normal hair in the Chicago area. *W. J. R. Camp and V. A. Gant,* Dept. Toxicology, Univ. of Illinois, Chicago.*
3. Observations on administration of BAL in selenium poisoning. *J. B. Belogorsky* and Donald Slaughter. Dept. Physiology and Pharmacology, Univ. of South Dakota School of Medicine, Vermillion.*
4. Chronic toxicity of phenylmercuric acetate and mercuric acetate in the rat. *Edwin P. Laug, O. G. Fitzhugh, Frieda Kunze* and A. A. Nelson. Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
5. Determination of beryllium in biological material. *T. Y. Toribara, W. F. Neuman, A. L. Underwood and M. W. Cucci (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*
6. Correlation between toxicity by inhalation and the physical properties of beryllium oxide. *R. H. Hall, S. Laskin, G. Sprague and B. H. Brown (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*
7. Further data on the feeding of beryllium salts to laboratory animals. *E. A. Maynard,* W. L. Downs,* J. K. Scott* and H. C. Hodge. Dept. Radiation Biology, Univ. of Rochester, N. Y.*
8. Preliminary studies of beryllium poisoning: II. One-year studies by intratracheal injection. *C. W. LaBelle, P. Morrow, C. Booth, F. Wescott and G. Melville (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*
9. Acute inhalation toxicity of beryllium sulfate in animals. *H. E. Stokinger, G. F. Sprague, R. H. Hall, J. K. Scott, F. A. Smith, B. H. Brown, and A. Pettengill (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*
10. Investigation of clinical criteria in beryllium poisoning. *C. J. Spiegl, F. A. Smith, L. J. LaFrance and J. W. Keating (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*
11. Distribution and excretion of beryllium sulphate using Be^7 as a tracer. *James K. Scott and William Neuman (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*
12. Metabolic studies in beryllium patients. *Joe W. Howland and Christine Waterhouse (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*

TUESDAY, APRIL 19, 4:15 P.M.

Pharmacology Business Meeting

LODGE ROOM A

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

Program on page 418

WEDNESDAY, APRIL 20, 9:00 A.M.

Autonomic Agents

LODGE ROOM A

C. H. Thienes, *Chairman*

1. A simple method for determining the degree of vascular sympatholytic action. *Raymond P. Ahlquist. Dept. Pharmacology, Univ. of Georgia School of Medicine, Augusta.*
2. Epinephrine and dibenamine in the alarm reaction. *Joseph Seifter, William E. Ehrich, Albert J. Begany* and George M. Hudyma.* Wyeth Institute of Applied Biochemistry, and the Graduate School of Medicine of the Univ. of Pennsylvania, Philadelphia.*

3. Responses to epinephrine and norepinephrine after dibenamine. *Walter A. Freyburger,* Luis R. Capo* and Gordon K. Moe. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
4. Adrenolytic activity of a number of N-phenyl-isopropyl-beta-haloethylamines. *Glenn E. Ullyot,* James Kerwin,* Edwin J. Fellows and Edward Macko.* Research Division, Smith, Kline & French Laboratories, Philadelphia, and Dept. Pharmacology, Temple Univ. School of Medicine, Philadelphia.*
5. Pharmacological studies on 2-(N-p′-tolyl-N-(m′-hydroxyphenyl)-aminomethyl-) imidazoline hydrochloride (C-7337). *M. R. Warren, R. A. Woodbury and J. H. Trapold.* Division of Pharmacology, Univ. of Tennessee, Memphis.*
6. Pharmacologic properties of 2-(N-p′-tolyl-N-(m′-hydroxyphenyl)-aminomethyl)- imidazoline hydrochloride (C-7337), a sympatholytic. *Elizabeth A. Herrold,* Anne Cameron,* Alfred Earl,* Frank Roth,* James Smith,* Nicoline Smith,* Elizabeth Sorensen* and Bradford N. Craver. Division of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.*
7. A study of the effect of adrenergic blocking agents on the vasopressor response to epinephrine. *David Russell* and Graham Chen. Research Laboratories, Parke, Davis & Co., Detroit.*
8. Pharmacologic and sensitizing properties of SY-28 when administered by ion transfer. *Arthur A. Rodriquez,* Jules H. Last and Adolph Rostenberg, Jr.* Depts. Physical Medicine, Pharmacology, Dermatology and Allergy Unit, Univ. of Illinois College of Medicine, Chicago.*
9. Cutaneous administration of adrenergic blocking agents by ion transfer. *Jules H. Last, Arthur A. Rodriquez* and Isadore Pitesky.* Depts. Pharmacology and Physical Medicine, Univ. of Illinois College of Medicine, Chicago.*
10. A new adrenergic blocking agent. *Francis G. Henderson* and K. K. Chen. The Lilly Research Laboratories, Eli Lilly & Co., Indianapolis.*
11. Adrenolytic activity of a series of N-(9-fluorenyl)-beta-chlorethylamines. *James Kerwin,* Glenn E. Ullyot,* Edwin J. Fellows and Edward Macko.* Research Division, Smith, Kline, & French Laboratories, and Dept. Pharmacology, Temple Univ. School of Medicine, Philadelphia.*
12. Ganglionic blocking action of diethylaminoethanol. *Byron B. Clark and Maurice M. Helpern.* Dept. Pharmacology, Tufts College Medical School, Boston, Mass.*

WEDNESDAY, APRIL 20, 9:00 A.M.

Anticonvulsants and Analgesics

ROOM 2

E. M. K. Geiling, *Chairman*

1. Pharmacological studies of phenacetylurea (Phenurone), an anticonvulsant drug. *Guy M. Everett (introduced by R. K. Richards). Dept. Pharmacology, Abbott Research Laboratories, North Chicago, Ill.*
2. Comparative anticonvulsant potencies of some phenylhydantoins and their corresponding phenylacetylureas. *Ewart A. Swinyard and James E. P. Toman. Depts. Pharmacy, Pharmacology and Physiology, Univ. of Utah College of Medicine and College of Pharmacy, Salt Lake City.*
3. Electroshock seizures in the hamster and their modification by antiepileptic drugs. *Edward H. Lanphier (introduced by Carl C. Pfeiffer). Dept. Pharmacology, Univ. of Illinois College of Medicine, Chicago.*
4. A comparative study of anticonvulsant activities of antiepileptic drugs by electroshock methods in cats, rats and mice. *Charles R. Ensor* and Graham Chen. Research Laboratories, Parke, Davis & Company, Detroit.*
5. Timed intravenous infusion of metrazol or strychnine in anticonvulsant testing. *Marshall J. Orloff,* H. L. Williams * and Carl C. Pfeiffer. Dept. Pharmacology, Univ. of Illinois College of Medicine, Chicago.*
6. Effect of tridione (3,3,5-trimethyl oxazolidine-2,4-dione) on the respiration of mouse brain. *J. D. Taylor,* R. K. Richards and G. M. Everett.* Dept. Pharmacology, Abbott Research Laboratories, North Chicago, Ill.*
7. Colour and crystal tests for the identification of methadon and isomethadon. *G. H. W. Lucas. Dept. Pharmacology, Univ. of Toronto, Canada.*
8. Respiration of human brain slices and the effects of morphine and methadone on oxygen uptake. *H. W. Elliott, V. C. Sutherland* and E. B. Boldrey,* Divisions of Pharmacology and Experimental Therapeutics and of Neurological Surgery, Univ. of California Medical School, San Francisco.*
9. Effect of cholinesterase inhibitors on the permeability of dog erythrocytes in vitro. *Margaret E. Greig and William C. Holland.* Dept. Pharmacology, Vanderbilt Univ. Medical School, Nashville, Tenn.*
10. Tolerance in rats to new synthetic analgesic drugs. *John R. Lewis (introduced by A. M. M. Lands). Biology Division, Sterling-Winthrop Research Institute, Rensselaer, N. Y.*
11. Studies on the fate of dl-methadon. *E. Leong Way, Chen-yū Sung* and William P. Mc-*

Kelway. Dept. Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

12. In vitro oxidation of morphine by tissue homogenates. *L. C. Abood and Ernest Kun (introduced by Julius M. Coon). Dept. Pharmacology, Univ. of Chicago, Ill.*
13. A comparison of heptazone (4,4-diphenyl-6-morpholinoheptanone-3-hydrochloride) with other analgesic drugs. *Lars Flataker* and Charles A. Winter. Merck Institute for Therapeutic Research, Rahway, N. J. (Physiol.)*

WEDNESDAY, APRIL 20, 1:30 P.M.

Cardiac Agents

LODGE ROOM A

R. P. Walton, *Chairman*

1. Relative potencies of various cinchona alkaloids in patients with auricular fibrillation. *Walter Modell, Samuel J. Shane,* Conrado Dayrit* and Harry Gold. Dept. Pharmacology, Cornell Univ. Medical College, the Cardiovascular Research Unit of the Beth Israel Hospital and the Cardiac Service of the Hospital for Joint Diseases, New York City.*
2. Xanthine studies: (1) effects of aminophyllin, 1,3-diethyl-8-bromoxanthine and 1,3-dimethyl-8-chloroxanthine on cardiac output. *D. M. Green, W. C. Bridges,* A. D. Johnson,* J. H. Lehmann,* F. Gray* and L. Field.* School of Medicine, Univ. of Washingington, Seattle.*
3. Potency and pharmacological actions of neriifolin, a glycoside of thevetia nerifolia. *Kalman C. Mezey (introduced by M. B. Chenoweth). Cesar Uribe-Piedrahita Research Laboratories, Bogota, Colombia.*
4. Induction of idioventricular rhythms by 1-1-1 trichlorethane and epinephrine. *Barbara R. Rennick,* S. Donald Malton,* G. K. Moe and M. H. Seevers. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
5. A single injection method for the assay of digitalis in pigeons. *H. A. Braun and L. M. Lusky.* Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
6. Respiratory activity in vitro of cardiac muscle of ouabainized dogs. *Albert Wollenberger (introduced by Otto Krayer). Dept. Pharmacology, Harvard Medical School, Boston, Mass.*
7. Cardiac-active principles in osage orange. *R. A. Waud, C. W. Gowdey* and J. S. Loynes.* Dept. Pharmacology, Univ. of Western Ontario, London, Canada.*
8. Comparative fluorimetric vs. biologic assays (human and animal) of fractions of digitalis purpurea. *Louis Sciarini* and William T. Salter. Dept. Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.*
9. Inotropic synergism between digitalis bodies and serum calcium. *William T. Salter, John Gemmel* and Louis Sciarini.* Dept. Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.*
10. Comparative effect of digitoxin and ouabain on auriculoventricular conduction in the dog heart. *Rafael Mendez and José Pisanty.* Dept. Physiology and Pharmacology of the National Institute of Cardiology, Mexico City.*
11. Failure of alpha tocopherol to influence chest pain in patients with heart disease. *Seymour H. Rinzler,* Hyman Bakst,* Zachary H. Benjamin,* Audrie L. Bobb* and Janet Travell. Cardiovascular Research Unit of Beth Israel Hospital, and the Dept. Pharmacology, Cornell Univ. Medical College, New York City.*
12. Relative local emetic activity of glycosides of the digitalis series in cats. *Romulo Guevara,* Walter Modell, Theodore Greiner,* Melvin Moore,* Abraham Blumer,* Harold Evans* and Harry Gold. Dept. Pharmacology, Cornell Univ. Medical College, New York City.*
13. Absence of antifibrillatory effect in quaternary nitrogen compounds *John E. Schults* and Joseph R. DiPalma. Depts. Physiology and Medicine, Long Island College of Medicine, Brooklyn, N. Y. (Physiol.)*
14. Antifibrillatory properties N-methyl-N-(3,4-dimethoxybenzyl) beta-(4-methoxyphenyl)-ethylamine hydrochloride. *Joseph R. DiPalma, Joseph J. Lambert,* Richard A. Reiss* and John E. Schults.* Depts. Physiology and Medicine, Long Island College of Medicine, Brooklyn, N. Y. (Physiol.)*

WEDNESDAY, APRIL 20, 1:30 P.M.

Drug Toxicity

PARLOR F

P. J. Hanzlik, *Chairman*

1. Studies on the storage of DDT in the fat of the rat. *Frieda Kunze,* A. A. Nelson, O. G. Fitzhugh and Edwin P. Laug. Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
2. Rate of disappearance of isomers of benzene hexachloride from fat depots in rats. *John P. Frawley* and O. Garth Fitzhugh. Division of Pharmacology, Food and Drug Adminis-*

tration, Federal Security Agency, Washington, D. C.

3. A comparison of the chronic toxicity of alpha, beta and gamma isomers of benzene hexachloride. *O. G. Fitzhugh, A. A. Nelson and Oma L. Holland.* Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
4. Studies in the pharmacologic action of chlordane. *E. F. Stohlman, W. T. S. Thorp and M. I. Smith. Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.*
5. Subacute respiratory toxicity of a new insecticide activator in 'aerosol bomb' mixture. *Robert O. Bauer and Walter W. Jetter (introduced by George L. Maison). Dept. Pharmacology, Boston Univ. School of Medicine, Boston.*
6. Effect of acetate donors on creatinine levels in blood and urine in dogs. *A. Kandel* and Maynard B. Chenoweth. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
7. Prevention of fluoroacetate poisoning by acetate donors. *Maynard B. Chenoweth, Edward B. Scott* and S. Louise Seki.* Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
8. Synergistic effect of sodium acetate and ethanol in antagonizing sodium fluoroacetate (1080) poisoning in mice. *W. W. Tourtellotte* and J. M. Coon. Univ. of Chicago Toxicity Laboratory and the Dept. Pharmacology, Univ. of Chicago, Ill.*
9. Effect of fluoroacetate on oxidative processes in frog muscle. *Donald A. Clarke,* James F. Toole* and Walter F. Riker. Dept. Pharmacology, Cornell Univ. Medical College, New York City.*
10. Effect of renal dysfunction on the urinary excretion of fluoride in the rabbit. *F. A. Smith and D. E. Gardner (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, N. Y.*
11. Influence of alpha-naphthylthiourea (ANTU) on iodine metabolism. *Richard U. Byerrum,* Kenneth W. Cochran* and Kenneth P. DuBois. Univ. of Chicago Toxicity Laboratory and the Dept. Pharmacology, Univ. of Chicago.*
12. Toxicity of N-p-chlorophenyldiazothiourea (Promurit) to mammals. *Kenneth W. Cochran* and Kenneth P. DuBois. Univ. of Chicago Toxicity Laboratory and the Dept. Pharmacology, Univ. of Chicago, Ill.*
13. An ultraviolet spectrophotometric method for the determination of benzene hexachloride. *Bernard Davidow and Geoffrey Woodard (introduced by R. L. Grant). Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C. (Biochem.)*

WEDNESDAY, APRIL 20, 1:30 P.M.

General Pharmacology

ROOM 2

E. R. Loew, *Chairman*

1. Uptake of bromsulphthalein by the liver of the rat II. Studies with radioactive bromsulphthalein (BSP). *John Krebs* and Ralph W. Brauer. Dept. Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans.*
2. A screening test for radiopaque drugs. *A. Light,* P. B. Dews,* J. Tornaben,* E. H. Lang,* Stata Norton* and Edwin J. de Beer. The Wellcome Research Laboratories, Tuckahoe, N. Y.*
3. A method for rapid graphic solution of time-per cent effect curves. *John T. Litchfield, Jr. Chemotherapy Division, Stamford Research Laboratories, American Cyanamid Company, Stamford, Conn.*
4. Separation by fluoroacetate of energy sources for smooth muscle contraction. *James R. Weeks* and Maynard B. Chenoweth. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
5. Relationship of chemical structure to biliary concentration in experimental cholecystography. *James O. Hoppe and S. Archer (introduced by A. M. Lands). Biology Division, Sterling-Winthrop Research Institute, Rensselaer, N. Y.*
6. Effects of podophyllotoxin and picropodophyllin on the intact intestine and salivary secretion of non-anesthetized dogs. *Margaret G. Kelly,* Aldo P. Truant* and Paul K. Smith. Dept. Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*
7. Effect of podophyllotoxin on enzymes and tissue metabolism. *Zelma Baker Miller, Clarke Davison* and Paul K. Smith. Dept. Pharmacology, George Washington Univ. School of Medicine, Washington, D. C. (Biochem.)*
8. Alteration of the activity of the human gastrointestinal tract with dihydroergocornine methanesulfonate (D. H. O. 180). *W. D. Bennett,* L. E. Morris* and O. Sidney Orth. Depts. Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison.*
9. Effect of some vasodilators and sedatives on gastric secretion. *Robert S. Schlesinger,* F. Steigmann and Leo L. Hardt.* Hektoen*

Institute for Medical Research of Cook County Hospital, and the Depts. Internal Medicine, University of Illinois College of Medicine and Stritch School of Medicine, Loyola Univ., Chicago

10. Evaluation of drugs possessing anti-dysmenorrhoea properties. *R. A. Woodbury and Paul P. C. Feng.* Division of Pharmacology, Univ. of Tennessee, Memphis.*
11. Influences of progesterone and testosterone on reactivity of rabbit myometrium to oxytocin and vasopressin. *Paul P. C. Feng* and R. A. Woodbury. Division of Pharmacology, Univ. of Tennessee, Memphis.*
12. Determination of diethylstilbestrol in urine. *R. S. Teague, Christian Wingard* and Albert E. Brown.* Dept. Pharmacology, Medical College of Alabama, Birmingham.*

WEDNESDAY, APRIL 20, 7:00 P.M.

Joint Session, The Biometric Society, Eastern North American Region, and The Pharmacological Society

BALLROOM—, BOOK-CADILLAC HOTEL

Biometrical Clinic on Pharmacological Problems
followed by general discussion

Chairman: Lloyd C. Miller, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

Discussants

1. C. I. Bliss, Connecticut Agricultural Experiment Station, New Haven, Conn.
2. A. E. Brandt, U. S. Atomic Energy Commission, New York City.
3. K. A. Brownlee, E. R. Squibb and Sons, New Brunswick, N. J.
4. D. B. Delury, Ontario Research Foundation, Toronto. Others to be announced later. The problems will be selected from those proposed by the pharmacologists for discussion at this session.

WEDNESDAY, APRIL 20, 9:30 P.M.

Federation Mixer

HOTEL STATLER BALLROOM

THURSDAY, APRIL 21, 9:00 A.M.

Autonomic Agents

LODGE ROOM A

C. C. Pfeiffer, *Chairman*

1. Pharmacology of a new cholinergic agent. *W. Clarke Wescoe* and Walter F. Riker. Dept. Pharmacology, Cornell Univ. Medical College, New York City.*
2. Effects of 3-acetoxy phenoxy trimethylammonium methylsulphate on neuromuscular function. *Walter F. Riker and W. Clarke Wescoe.* Dept. Pharmacology, Cornell Univ. Medical College, New York City.*
3. Actions of calcium and tetraethylammonium ions on the rat diaphragm preparation. *Charles J. Kensler (introduced by McKeen Cattell). Dept. Pharmacology, Cornell Univ. Medical College, New York City.*
4. Effect of tetraethylammonium on the response of the isolated rabbit heart to nicotine. *J. M. Coon, P. R. Salerno* and L. E. Ellinwood.* Dept. Pharmacology, Univ. of Chicago, Ill.*
5. Effects of tetraethylammonium chloride on pain thresholds in man. *Ralph R. Sonnenschein,* Elizabeth H. Jenney* and Carl C. Pfeiffer. Depts. Pharmacology and Clinical Science, Univ. of Illinois College of Medicine, Chicago.*
6. Ganglionic blocking action of "dibutoline." *Carl C. Gruhzit* and Gordon K. Moe. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
7. Mechanism of action of anticholinesterase. *Theodore Koppanyi and Alexander G. Karczmar.* Dept. Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*
8. Skin penetration of radioactive anticholinesterases—a direct quantitative method of study. *E. Ross Hart, Joseph H. Fleisher* and Amedeo S. Marrazzi. Toxicology Section, Medical Division, Army Chemical Center, Md.*
9. Reaction of cholinesterase with diisopropyl fluorophosphate containing radioactive phosphorus. *Harry O. Michel* and Stephen Krop. Biochemistry and Pharmacology Sections, Medical Division, Army Chemical Center, Md.*
10. Studies on the inhibition of the cholinesterase activity of human blood plasma. II. Studies with hexaethyltetrapolyphosphate containing C^{14}. *Ralph W. Brauer and Rita L. Pessotti.* Dept. Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans.*
11. Pharmacologic and toxicologic data concerning diethylaminoethyl ester of 1-phenyl-cyclopentane-1-carboxylic acid hydrochloride (Parpanit). *Charles P. Kraatz,* Charles M. Gruber, Jr., Hubert L. Shields* and Charles M. Gruber. Dept. Pharmacology, Jefferson Medical College, Philadelphia.*
12. Effect of diethylaminoethyl ester of 1-phenyl-cyclopentane-1-carboxylic acid hydrochloride, "Parpanit," on decerebrate rigidity, spinal reflexes and skeletal muscle *Charles*

M. Gruber, Charles P. Kraatz, Charles M. Gruber, Jr. and Joan E. Copeland.* Dept. Pharmacology, Jefferson Medical College, Philadelphia.*

THURSDAY, APRIL 21, 9:00 A.M.

Curare and Curariform Agents

ROOM 2

H. R. Hulpieu, *Chairman*

1. Effect of myanesin on extrapyramidal facilitatory and inhibitory systems. *E. Henneman,* A. Kaplan* and K. Unna. Depts. Psychiatry and Pharmacology, Univ. of Illinois College of Medicine, Chicago.*
2. Anticonvulsive properties of myanesin. *K. Unna and A. Kaplan.* Dept. Pharmacology, Univ. of Illinois College of Medicine, Chicago.*
3. Preparation and pharmacological properties of the acid succinate of myanesin. *Richard F. Riley and F. M. Berger (introduced by Harold C. Hodge). Depts. Pharmacology and Pediatrics, Univ. of Rochester, N. Y.*
4. Oral activity of analogues of 3-ortho-toloxy-1, 2-propanediol. *John C. Burke, Richard K. Thomas, G. Lee Hassert, Jr. and Rita J. Wilkins (introduced by Charles R. Linegar). Pharmacological Development Division, E. R. Squibb & Sons, New Brunswick, N. J.*
5. Paralysing action of 2-methyl, 2-n-amyl-4-hydroxymethyl-1,3-dioxolane (glyketal). *F. M. Berger (introduced by Arnold D. Welch). Univ. of Rochester School of Medicine, N. Y.*
6. Elimination of d-tubocurarine in the rat. *E. F. Van Maanen (introduced by Otto Krayer). Dept. Pharmacology, Harvard Medical School, Boston.*
7. Synthetic curare compounds. IV. N-methylberbamine iodide and N-methyl-isotetrandine. *David Fielding Marsh and Donald A. Herring.* Dept. Pharmacology, West Virginia Univ. School of Medicine, Morgantown.*
8. Curariform action of decamethylene-1, 10-bis-trimethylammonium bromide. *Julio C. Castillo* and Edwin J. de Beer. The Wellcome Research Laboratories, Tuckahoe, N. Y.*
9. "Curare-like" action of synthetic quaternary derivatives of stilbene. *Bernard P. McNamara and Teresa Silverman.* Medical Division, Army Chemical Center, and Univ. of Maryland, School of Pharmacy, Baltimore.*

THURSDAY, APRIL 21, 9:00 A.M.

General Pharmacology

ROOM 2

I. Starr, *Chairman*

1. Study of coca addiction. *Carlos Gutierrez-Noriega. Instituto de Farmacologia y Terapeutica, Univ. Nacional Mayor de San Marcos, Lima, Peru.*
2. Effects of inhalation of high concentrations of carbon dioxide. *Thomas M. Parry,* Joseph N. Spencer,* William B. Draper, Richard W. Whitehead and Robert L. Arends.* Dept. Physiology and Pharmacology, Univ. of Colorado Medical Center, Denver.*
3. Pharmacology and toxicology of thiodipropionic acid and its dilauryl and distearyl esters. *Lloyd W. Hazleton and Rebecca C. Hellerman.* Hazleton Laboratories, Falls Church, Va.*
4. Use of C^{14} in studies of the metabolism of plant products. *F. E. Kelsey, Dept. Pharmacology, Univ. of Chicago, Ill.*
5. Distribution effect and fate of oil aerosol particles after retention in the lungs of mice. *Milton Shoshkes, William G. Banfield, Jr. and S. Joseph Rosenbaum (introduced by Amedeo S. Marrazzi). Toxicology, Pathology and Aerosol Sections, Medical Division, Army Chemical Center, Md.*
6. Renal clearance of iodide in the dog. *Douglas Shepard Riggs. Dept. Pharmacology, Harvard Medical School, Boston.*
7. In vivo action of morphine, urethane and phenobarbital on the glycogen synthesis from glucose in the rat liver. *Ernest Kun and L. G. Abood (introduced by E. M. K. Geiling). Dept. Pharmacology, Univ. of Chicago.*
8. Absorption and distribution of isopropyl alcohol. *Joan Wax,* Fred W. Ellis and Arnold J. Lehman. Dept. Pharmacology, School of Medicine, Univ. of North Carolina, Chapel Hill.*
9. Comparative studies of dihydroergot derivatives. *R. T. Capps,* H. M Suckle* and O. Sidney Orth. Depts. Pharmacology and Neurosurgery, Univ. of Wisconsin Medical School, Madison.*
10. Comparative activities of five antispasmodics as adjudged by actions on salivation and nictitating membrane of cat, on vagal block and antagonism to mecholyl. *B. Craver, W. Barrett,* A. Cameron,* A. Earl* and F. Roth.* Division of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.*

11. Action of synthetic antispadmodics and local anesthetics on human gastric motility. *George P. Child* and Robert A. Woodbury. Dept. Physiology and Pharmacology, Albany Medical College, Albany, N. Y., and Dept. Pharmacology, Univ. of Tennessee Medical College, Memphis.*

THURSDAY, APRIL 21, 1:30 P.M.

Cardiac Agents and Diuretics

LODGE ROOM A

T. Sollmann, *Chairman*

1. Cardiac and calorigenic actions of dicumarol and dinitrophenol. *R. P. Walton, C. B. Hanna,* R. P. Prystowsky* and J. S. Leary.* Dept. Pharmacology, Medical College of South Carolina, Charleston.*
2. A study of cyclic and noncyclic hydrocarbons on cardiac automaticity. *C. Jelleff Carr and John C. Krantz, Jr. Dept. Pharmacology, Univ. of Maryland School of Medicine, Baltimore.*
3. Effect of quinidine in cyclopropane and cyclopropane-epinephrine induced arrhythmias. *R. A. Huggins,* R. A. Morse,* D. W. Chapman,* C. A. Handley and L. F. Schuhmacher.* Depts. Pharmacology, Medicine and Anesthesiology, Baylor Univ. College of Medicine, Houston, Texas.*
4. Studies on radioactive digitoxin (digitalis lanata). *T. Eugene Kimura* and E. M. K. Geiling. Dept. Pharmacology, Univ. of Chicago.*
5. Biosynthesis of radioactive bufagin containing C^{14}. *John Doull,* Kenneth P. DuBois and E. M. K. Geiling. Dept. Pharmacology, Univ. of Chicago.*
6. Inhibitory action of bufagin on carbohydrate oxidation. *Kenneth P. DuBois, John Doull* and E. M. K. Geiling. Dept. Pharmacology, Univ. of Chicago.*
7. Method for assaying potential coronary vasodilators. *A. Cameron,* E. Sorensen* and B. Craver. Division of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.*
8. Inhibition of myocardial reactions to epinephrine and norepinephrine by nitroglycerine. *W. Raab and Eugene Lepeschkin (introduced by F. J. Sichel). Division of Experimental Medicine, Univ. of Vermont College of Medicine, Burlington.*
9. A comparative study of the local toxic action of thiomerin, mercuzanthin and mercuhydrin. *Harry Taube,* Robert A. Lehman and E. E. King.* Dept. Therapeutics, New York Univ. College of Medicine, New York City.*
10. Effect of mercurial diuretics on the succinic dehydrogenase system of the kidney. *C. A. Handley. Dept. Pharmacology, Baylor Univ. College of Medicine, Houston, Texas.*
11. Subcutaneous use of thiomerin, a new mercurial diuretic for treatment of congestive heart failure. *Robert C. Batterman, David Unterman* and Arthur C. DeGraff. Dept. Therapeutics, New York Univ. College of Medicine, New York City.*
12. Pharmacology of thiomerin. *Robert A. Lehman and E. E. King.* Dept. Therapeutics, New York Univ. College of Medicine, New York City.*
13. Myocardial insufficiency and its management with especial reference to a new and safe mercurial diuretic. *George R. Herrmann, John W. Chriss,* E. H. Schwab,* Milton R. Hejtmancik* and Paul M. Sims.* Univ. of Texas, Medical Branch, Galveston (Physiol.)*
14. Diuretic response of normal and edematous dogs to intravenous administration of succinate and fumarate sodium salts. *Elizabeth O'Leary and Samuel A. Corson. Dept. Physiology, School of Medicine, Howard Univ., Washington, D. C. (Physiol.)*

THURSDAY, APRIL 21, 4:15 P.M.

Pharmacology Business Meeting

LODGE ROOM A

THURSDAY, APRIL 21, 7:00 P.M.

Pharmacology Dinner

MASONIC TEMPLE, COLONADE ROOM

THURSDAY, APRIL 21, 1:30 P.M.

General Pharmacology

ROOM 2

J. H. Weatherby, *Chairman*

1. Hypotensive activity of fractions from green hellebore. *J. W. Stutzman, George L. Maison and G. W. Kusserow.* Dept. Pharmacology, Boston Univ. School of Medicine, and Product Development Dept., Rexall Drug Company, Los Angeles.*
2. Fate of dicoumarol in man. *Murray Weiner,* Julius Axelrod,* Shepard Shapiro* and Bernard B. Brodie. Depts. Biochemistry and Medicine, New York Univ. College of Medicine, and New York Univ. Medical and Re-*

search Services, Goldwater Memorial Hospital, New York City.

3. Depressor action of nitrate esters of alkyl glycollates. *Joseph G. Bird,* Harry K. Iwamoto, John B. Harmon* and John C. Krantz, Jr. Dept. Pharmacology, Univ. of Maryland School of Medicine, Baltimore.*
4. Studies on the cardiovascular action of magnesium sulfate solutions. *James P. Hensen (introduced by R. P. Ahlquist). Dept. Pharmacology, Univ. of Georgia School of Medicine, Augusta.*
5. Paper chromatography of flavonoid pigments. II. The separation and quantitative estimation of rutin and quercetin. *Thomas B. Gage and Simon H. Wender (introduced by Arthur A. Hellbaum). Chemistry Dept., Univ. of Oklahoma, Norman.*
6. Influence of orally administered rutin on adrenal ascorbic acid storage in guinea pigs. *Anthony M. Ambrose and Floyd DeEds. Pharmacology Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Albany, Calif.*
7. Antihistaminic properties of the β-haloalkylamine (dibenamine) series of adrenergic blocking agents. *Mark Nickerson and Frances B. Harris.* Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*
8. Action of diphenhydramine (benadryl) in reversing the "epinephrine reversal" of blood pressure. *Graham Chen and David Russell.* Research Laboratories, Parke, Davis & Company, Detroit.*
9. Pharmacological evaluation of trimeton, 1-phenyl-1-(2-pyridyl)-3-N, N-dimethylpropylamine. *Richard Tislow and Annette LaBelle with the assistance of Alois J. Makovasky, Mary Ann Griffith Reed, Margaret D. Cunningham, Jane F. Emele, Arnold Grandage and Ruth J. M. Roggenhofer (introduced by H. B. Haag). Biological Research Laboratories, Schering Corporation, Bloomfield, N. J.*
10. Relation of 4-methyl and 4-ethyl substituted imidazoles to: 1. histaminic action; 2. antagonism by antihistaminics. *W. Barrett,* J. Feickert* and B. Craver. Division of Macrobiology, Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.*
11. An evaluation of several types of drugs and combinations against anaphylaxis in guinea pigs. *Barbara B. Brown* and Harold W. Werner. Pharmacology Dept., Research Laboratories, The Wm. S. Merrell Company, Cincinnati.*

THURSDAY, APRIL 21, 4:15 P.M.

Pharmacology Business Meeting

LODGE ROOM A

THURSDAY, APRIL 21, 7:00 P.M.

Pharmacology Dinner

MASONIC TEMPLE

COLONADE ROOM

FRIDAY, APRIL 22, 9:00 A.M.

Autonomic Agents

LODGE ROOM A

M. H. Seevers, *Chairman*

1. Action of cholinesterase inhibitors on the isolated rabbit ileum. *P. R. Salerno* and J. M. Coon. Dept. Pharmacology, Univ. of Chicago.*
2. Anti-anemic agents and cholinesterase activity. *John Emerson Davis. Pharmacological Laboratory, Univ. of Arkansas, Little Rock.*
3. Antagonism of cinchona alkaloids to the circulatory effects of intravenous epinephrine. *Edwin P. Hiatt. Dept. Physiology, Univ. of North Carolina, Chapel Hill. (Physiol.).*
4. Central hyperglycemic action of 1-(3,4-dihydroxyphenyl) 2-isopropyl-aminoethanol (Isuprel). *A. Leimdorfer. Dept. Pharmacology, Loyola Univ., Chicago. (Physiol.)*
5. Comparison of effects of a new ganglionic blocking agent and a new sympatholytic. *K. S. Grimson, J. R. Chittum* and F. H. Longino*. Dept. Surgery, Duke Univ., Durham, N. C. (Physiol.)*
6. Effect of 933F on the action of dibenamine. *John C. Seed and Elizabeth A. MacKay (introduced by J. H. Wills). Pharmacology Section, Medical Division, Army Chemical Center, Md. (Physiol.)*
7. Vasomotor side effects of two new amines which primarily depress intestinal motility. *J. R. Chittum, F. H. Longino and B. H. Metcalf (introduced by K. S. Grimson). Dept. Surgery, Duke Univ., Durham, N. C. (Physiol.)*
8. Effect of N, N-dibenzyl-beta-chloroethylamine (dibenamine) on blood sugar response to insulin and epinephrine. *R. Lorimer Grant. Division Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C. (Biochem.)*

9. A new ganglionic blocking agent. *Lowell O. Randall, William G. Peterson* and G. Lehmann. Pharmacology Dept., Hoffmann-La-Roche, Inc., Nutley, N. J. (Biochem.).*
10. Interaction between di-isopropyl fluorophosphate (DFP) and proteins of brain tissue. *Bernard J. Jandorf and Priscilla D. McNamara.* Biochemistry Section, Medical Division, Army Chemical Center, Md. (Biochem.).*
11. Distribution of radiophosphorus in rabbit tissues after injection of labelled di-isopropyl fluorophosphate (DFP). *Priscilla D. McNamara* and Bernard J. Jandorf. Biochemistry Section, Medical Division, Army Chemical Center, Md. (Biochem.).*

FRIDAY, APRIL 22, 9:00 A.M.

Drug Toxicity

LODGE ROOM D

A. S. Marrazzi, *Chairman*

1. An experimental study of the toxicity of ozone under simulated industrial conditions. *M. G. Gray. Laboratories of Arthur D. Little, Inc., Cambridge, Mass.*
2. Toxicity to rats of diallyl acetic acid and other compounds containing the allyl radical. *Ernest C. Hagan*, Geoffrey Woodard* and Arthur A. Nelson. Division Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
3. Comparative percutaneous toxicity of 3-mercapto-1, 2-propanediol (thioglycerol) and ammonium thioglycollate. *John H. Draize, Elsie Alvarez* and Marie Woodard.* Division Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
4. Chronic effects of large doses of acetanilid in dogs and rats. *Alexander G. Karczmar (introduced by Theodore Koppanyi). Dept. Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*
5. Toxic effects of parenterally administered cetylpyridinium chloride. *Charles R. Thompson* and Harold W. Werner. Pharmacology Dept., Research Laboratories, Wm. S. Merrell Co., Cincinnati.*
6. Rate of removal of alcohol in acutely intoxicated chronic alcoholics. *H. W. Smith and R. G. Bell (introduced by J. K. W. Ferguson). Dept. Pharmacology, Univ. of Toronto and Shadowbrook Health Foundation.*
7. Observations on the effect of chemical configuration on the edema-producing potency (EPP) of acid, aldehydes, ketones and alcohols. *P. S. Larson, J. K. Finnegan and H. B. Haag. Dept. Pharmacology, Medical College of Virginia, Richmond.*
8. Actions of 2,6-diaminopurine in mice, rats, and dogs. *Frederick S. Philips and J. B. Thiersch.* Division Exptl. Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.*
9. Action of 4-amino, N, 10-methyl, pteroylglutamic acid in mice, rats, and dogs. *F. C. Ferguson, Jr.,* J. B. Thiersch* and F. S. Philips. Dept. Pharmacology, Cornell Univ. Medical College, and Division of Exptl. Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.*
10. Effect of intravenous 'Tween' solutions in various animals and man. *John C. Krantz, Jr. and Joseph G. Bird.* Dept. Pharmacology, Univ. of Maryland School of Medicine, Baltimore.*
11. Experimental safety of sorbitan monostearate and its polyoxyethylene derivatives. *Sidney G. Page, Jr. (introduced by Harvey B. Haag). Medical College of Virginia, Richmond.*
12. Purification of the toxic principle of agene-treated gluten for pharmacological study. *Jack L. Radomski, Geoffrey Woodard and Henry N. Fuyat (introduced by Arnold J. Lehman). Division Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
13. Some metabolic changes observed in seizures induced by certain agene-treated protein in dogs. *Julius Belford * and D. D. Bonnycastle. Laboratory of Pharmacology, Yale Univ., New Haven.*

FRIDAY, APRIL 22, 9:00 A.M.

Antibiotics, Antiseptics and Chemotherapeutic Agents

ROOM 2

M. G. Mulinos, *Chairman*

1. Relative merits of commonly used sulfonamide drugs as components of mixtures. *David Lehr. Dept. Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.*
2. Studies on the in vitro oxidation of oxophenarsine hydrochloride. *Ray E. Green (introduced by A. L. Tatum). Dept. Pharmacology, Univ. of Wisconsin Medical School, Madison.*
3. Therapeutic efficiency of various dosage schedules of oxophenarsine in experimental

trypanosomiasis. *Harry A. Walker, Susanne Wilson* and A. P. Richardson. Dept. Pharmacology, Emory Univ. School of Medicine, Ga.*

4. Coenzyme 1-quinine relationships in bacterial growth and metabolism. *R. M. Featherstone and J. R. Porter.* Depts. Pharmacology and Bacteriology, College of Medicine, State Univ. of Iowa, Iowa City.*
5. Effect of antimalarial drugs on anaerobic glycolysis (acid production) in tissue extracts. *Ralph Jones, Jr. (introduced by Carl F. Schmidt). Dept. Research Medicine, Univ. of Pennsylvania, Philadelphia.*
6. Chemotherapeutic effect of 8-amino quinolines in schistosoma mansoni infections in mice. *Raymond N. Bieter, Elizabeth M. Cranston, Wayne Chadbourn,* Ashton C. Cuckler,* Dominic DeGusti,* W. W. Becklund* and Harold N. Wright. Dept. Pharmacology, Univ. of Minnesota Medical School, Minneapolis.*
7. Efficacy and toxicity of Simaroubidin in experimental amoebiasis. *Ashton C. Cuckler and Carl C. Smith (introduced by Hans Moliter). Merck Institute for Therapeutic Research, Rahway, N. J.*
8. Studies on furan compounds: toxicity and pharmacological action of furfuryl alcohols. *John E. Gajewski and William R. Alsdorf (introduced by C. H. Hine). Naval Medical Research Institute, Bethesda, Md.*
9. Antiseptic properties of certain mercurial diuretics. *S. Anderson Peoples and Ann McCormick.* Division of Veterinary Science, Univ. of California, Davis.*
10. Pharmacological studies on actidione. *Andres Goth and Fabian J. Robinson.* Dept. Physiology and Pharmacology, Southwestern Medical College, Dallas, Texas.*
11. Indirect in vitro action of antibiotics in comparison with activity of accepted amebacides. *John L. Bradin, Jr., and Eder L. Hansen (introduced by Hamilton H. Anderson). Division of Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco.*
12. Toxicology and pharmacology of lupulon. *Yin-ch'ang Chin* and Hamilton H. Anderson. Division of Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco.*
13. Absorption of sulfamylon. *E. W. McChesney, M. E. Auerbach* and H. W. Eckart.* Sterling-Winthrop Research Institute, Rensselaer, N. Y. (Physiol.).*

FRIDAY, APRIL 22, 1:30 P.M.

Anesthetics, Local and General

LODGE ROOM A

C. J. Carr. *Chairman*

1. Influence of ethyl chloride spray on deep pain and ischemic contraction of skeletal muscle. *J. Travell and S. H. Rinzler.* Dept. of Pharmacology, Cornell Univ. Medical College, and Cardiovascular Research Unit of Beth Israel Hospital, New York City.*
2. Effect of local anesthetics on respiration of brain homogenates. *Daniel T. Watts, (introduced by C. L. Gemmill). Dept. of Pharmacology, Univ. of Virginia Medical School, Charlottesville.*
3. Comparison of local anesthetic activity of certain aliphatic amines. *Herbert W. Taylor, Jr.,* Edwin J. Fellows, Edward Macko* and Glenn E. Ullyot.* Research Division, Smith, Kline and French Laboratories, Philadelphia, and Dept. Pharmacology, Temple Univ., School of Medicine, Philadelphia.*
4. Local anesthetic activity of a series of aminoalkoxy isoquinolines. *Edwin J. Fellows and Edward Macko.* Dept. Pharmacology, Temple Univ. School of Medicine, Philadelphia.*
5. Effect of epinephrine on the toxicity of procaine. *Versa V. Cole and H. R. Hulpieu. Dept. Biochemistry and Pharmacology, Indiana Univ., Indianapolis.*
6. Study of cocaine-barbiturate antagonism. *John E. Steinhaus (introduced by A. L. Tatum). Dept Pharmacology, Univ. of Wisconsin Medical School, Madison.*
7. Further studies on competitive inhibition of convulsions in guinea pigs. *R. K. Richards and K. E. Kueter.* Dept. Pharmacology, Abbott Research Laboratories, North Chicago.*
8. Some effects of certain central nervous system depressants and stimulants on intermediary metabolism of brain. *F. E. Shideman. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
9. Local anesthetic action of some thiazolidone derivatives. *F. P. Luduena and James O. Hoppe.* Biology Division, Sterling-Winthrop Research Institute, Rensselaer, N. Y.*
10. Effects of excess carbon dioxide in clinical anesthetic atmospheres upon cardiac automaticity and liver function. *O. Sidney Orth, Ralph M. Waters, Lucien E. Morris,* J. L. Sims* and Robert T. Capps.* Depts. Pharmacology, Anesthesiology and Internal Medicine, Univ. of Wisconsin Medical School, Madison.*
11. A method for the estimation of chloroform in small quantities of blood. *Lucien E. Morris*

and Evan L. Frederickson (introduced by O. Sidney Orth). Depts. Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison.

12. Trichloroethanol as a metabolic product of trichloroethylene. *Thomas C. Butler. Dept. Pharmacology and Experimental Therapeutics, The Johns Hopkins Univ., Baltimore.*
13. Anesthesia with methylal in mice and dogs. *Robert W. Virtue. Division Anesthesiology, Dept. Surgery State Univ. of Iowa, Iowa City. (Biochem.)*

FRIDAY, APRIL 22, 1:30 P.M.

General Pharmacology

ROOM 10

A. D. Welch, *Chairman*

1. Comparative effects of adenosine, isoguanosine and related compounds on smooth muscle. *Paul L. Ewing, Fritz Schlenk* and G. A. Emerson. Dept Pharmacology, Univ. of Texas Medical Branch, Galveston and Dept. Bacteriology, Iowa State College, Ames.*
2. Protective effect of certain drugs on epinephrine-induced pulmonary edema. *Clement A. Stone* and Earl R. Loew. Dept. Physiology, Boston Univ. School of Medicine.*
3. Relation between chemical constitution and estrogenic action in a group of azomethines. *H. H. Keasling and F. W. Schueler (introduced by E. G. Gross). College of Pharmacy and Dept. Pharmacology, College of Medicine, State Univ. of Iowa, Iowa City.*
4. Comparison of effects of injection of pyrogenic solutions by intravenous and intramuscular routes. *William D. Harkness* and Bert J. Vos. Division Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
5. Reactions of hemoglobin with aliphatic and inorganic nitrate and nitrite esters. *Harold Wooster* and F. William Sunderman. Mellon Institute, Pittsburgh and Cleveland Clinic, Cleveland. (Biochem.)*
6. The nephrotoxic action of a cyanine dye, 1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido- 2′-cyanine chloride. *Lawrence Peters and Aeme Higashi.* Dept. Pharmacology, School of Medicine, Western Reserve Univ., Cleveland.*
7. Maintenance of *T. equiperdum* in vitro at 38°C. *William Cantrell (introduced by E. M. K. Geiling). Dept. Pharmacology, Univ. of Chicago.*
8. Effect of antihistaminic drugs upon trained rats. *Charles A. Winter and Lars Flataker.* Merck Institute for Therapeutic Research, Rahway, N. J. (Physiol.)*
9. Comparison of pharmacological activities of several antihistaminic agents. *Robert T. Ford, Shirley L. McHugh, Virginia M. O'Rourke, Dorothy J. Nixon and Gerald Q. O'Neill (introduced by Karl H. Beyer). Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pennsylvania. (Physiol.)*
10. A new chemical method for the determination of histamine in biological materials. *Floyd C. McIntire, Muriel Sproul and Frances B. White (introduced by R. K. Richards). Biochemistry, Abbott Research Laboratories, North Chicago. (Physiol.).*
11. Effect of coffee on the growth of white rats. *Arthur H. Steinhaus and Aaron Spitzer.* George Williams College Laboratory for Physiologic Research in Physical Education, Hyde Park, Ill. (Physiol.)*

FRIDAY, APRIL 22, 1:30 P.M.

Drug Toxicity

ROOM 2

J. T. Litchfield, *Chairman*

1. Toxicity of hydroquinone for laboratory animals. *Geoffrey Woodard, E. Conway Hagan and Jack L. Radomski (introduced by Bert J. Vos). Division Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*
2. Acute toxicity of hydroquinone. *Harold C. Hodge, Elliott A. Maynard* and James H. Sterner.* Division Pharmacology and Toxicology, Dept. Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, and Eastman Kodak Company, Rochester, N. Y.*
3. Comparison of the acute toxicity of hydroquinone and some related di and trihydroxy benzenes. *James H. Sterner,* Stanley Ames* David W. Fassett. Eastman Kodak Company, Rochester, N. Y.*
4. Chronic toxicity studies on hydroquinone. *N. R. Brewer* and A. J. Carlson, Physiology Laboratory, Univ. of Chicago. (Physiol.).*
5. LD_{50} studies on hydroquinone. *Helen Burson,* N. R. Brewer* and A. J. Carlson. Physiology Laboratory, Univ. of Chicago. (Physiol.).*
6. Toxicity studies on antioxidants—butylated hydroxyanisole and hydroquinone. *O. H. M. Wilder and H. R. Kraybill (introduced by A. J. Carlson. American Meat Institute Foundation, Univ. of Chicago (Physiol.).*
7. A method for determining hydroquinone ex-

cretion in the urine. *David W. Fassett. Laboratory of Industrial Medicine, Eastman Kodak Company, Rochester, N. Y.*

8. Certain physico-chemical and toxic properties of phenol-camphor preparations. *Wm. B. Deichmann and T. Miller.* Division Pharmacology, Dept. Physiology and Pharmacology, Albany Medical College, Albany.*
9. Ratio of amino acid nitrogen to creatinine in the urine as a sensitive diagnostic test for uranium poisoning in the rabbit. *H. Berke and A. Rothstein (introduced by H. C. Hodge). Dept. Radiation Biology, Univ. of Rochester, Rochester, N. Y.*
10. Fate of mercury in the rat following administration as mercuric acetate and phenylmercuric acetate. *Claudia S. Prickett,* Edwin P. Laug and Frieda Kunze.* Division Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C. (Biochem.).*
11. Toxicity of tetramethyl succinonitrile and the antidotal effects of thiosulfate, nitrite and barbiturates. *R. N. Harger and H. R. Hulpieu. Dept. Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis. (Biochem.).*
12. Effect of arsenic on the metabolism of selenium. *Harlan L. Klug,* Donald F. Petersen* and Alvin L. Moxon. Chemistry Dept., South Dakota Agricultural Experiment Station, Brookings (Biochem.).*

Papers Read by Title

1. Effect of morphine sulfate on cerebral blood flow and metabolism in man. *Benedict E. Abreu, Henry W. Elliott, Violette C. Sutherland*, Lester Margolis* and Alexander Simon*. Divisions of Pharmacology and Experimental Therapeutics and of Psychiatry, Univ. of California Medical School, and Langley Porter Clinic, State Dept. of Mental Hygiene, San Francisco.*
2. Sympatholytic action of dibenamine. *Raymond P. Ahlquist. Dept. Pharmacology, Univ. of Georgia School of Medicine, Augusta.*
3. Survival of plasmodium cathemerium sporozoites in hen blood with addition of canary blood cells. *Harry Beckman. Marquette Univ. School of Medicine, Milwaukee.*
4. Studies on the effect of a number of aralkylamines on tyramine oxidation by amine oxidase. *Frederick Bernheim and Edwin J. Fellows. Duke Univ. School of Medicine, Durham, N. C., and Temple Univ. School of Medicine, Philadelphia.*
5. Relation of chemical structure to action of hyaluronidase inhibitor. *B. Calesnick and K. R. Beutner (introduced by R. Beutner). Dept. Pharmacology, Hahnemann Medical College, Philadelphia, and Keeley Institute, Dwight, Ill.*
6. N-(p-arsenoso-benzyl)-glycinamide HCl as an amebacide, *in vitro* and *in vivo* in macaques. *Y. T. Chang and R. K. Reed (introduced by Hamilton H. Anderson). Division of Pharmacology and Exptl. Therapeutics, Univ. California Medical School, San Francisco.*
7. Comparison of the cardiac action of thevebioside and neriifolin with thevetin. *K. K. Chen, Robert C. Anderson* and Francis G. Henderson*. The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis.*
8. Effect of local anesthetics on the motility of isolated rabbit ileum. *George P. Child (introduced by Robert A. Woodbury). Dept. Physiology and Pharmacology, Albany Medical College, Albany, N. Y.*
9. Comparative muscle-relaxing activity of seven glycerol ethers on isolated nerve-muscle preparations. *Harold Davis,* Charles H. Hine and Frank J. Murphy*. Division Pharmacology and Experimental Therapeutics and Dept. Anesthesiology, Univ. of California Medical School, San Francisco.*
10. Antiepileptic action of marihuana-active substances. *Jean P. Davis and H. H. Ramsey (introduced by Louis S. Goodman). Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, and Utah State Training School, American Fork, Utah.*
11. A fatal case of bartonella canis which was treated with penicillin. *John Emerson Davis. Pharmacological Laboratory, Univ. of Arkansas, Little Rock.*
12. Pentobarbital hypnosis in guinea pigs on ascorbic acid-deficient diet. *B. De Boer and K. O. De Boer*. Dept. Pharmacology, St. Louis Univ. School of Medicine.*
13. Experimental skin sensitization to sulfonamides and penicillin. *Robert H. Dreisbach. Dept. Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco.*
14. Bromate distribution in blood. *A. L. Dunn* and A. R. McIntyre. Dept. Physiology and Pharmacology, Univ. of Nebraska College of Medicine, Omaha.*
15. Biliary excretion and intestinal reabsorption of C^{14}-labeled methadone hydrobromide. *L. L. Eisenbrandt, I. A. Abdou and T. K. Adler (introduced by H. W. Elliott). Division Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco.*
16. Metabolism of isopropyl alcohol by the perfused liver. *Fred W. Ellis. Dept. Pharmacology, Univ. of North Carolina, Chapel Hill, N. C.*

17. Chemotherapeutic efficacy of nitrofurazone (furacin) in experimental trypanosomiasis of the mouse. *N. J. Giarman*, B. A. Rubin* and E. L. McCawley. Laboratories of Pharmacology and Bacteriology, Yale Univ. School of Medicine, New Haven.*

19. A new potent analgesic agent. *E. G. Gross, M. A. Brotman*, S. F. Nagyfy*, W. W. Sawtelle* and L. L. Zager*. Depts. Pharmacology, Anesthesiology, Obstetrics and Gynecology, Urology and Surgery, State Univ. of Iowa, Iowa City.*

20. Evaluation of the amebacidal action of agents inhibitory to associated bacteria *in vitro*. *Eder L. Hansen (introduced by Hamilton H. Anderson). Division Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco.*

21. A rapid method for the determination of insulin in blood and urine. *Aeme Higashi* and Lawrence Peters. Dept. Pharmacology, Western Reserve Univ. School of Medicine, Cleveland.*

22. Lack of antagonism of methionine to barbiturates. *J. Eugene Hill*, P. L. Ewing, G. A. Emerson and Melville Sahyun. Dept. Pharmacology, Univ. of Texas Medical Branch, Galveston, and The Sahyun Laboratory, Santa Barbara, Calif.*

23. Failure to demonstrate biological competition between hypnotic and non-hypnotic barbiturates. *Walter W. Jetter, Robert P. Brittain and Mary K. Nuter (introduced by George L. Maison). Dept. Legal Medicine, Harvard Medical School, and the Massachusetts Dept. Mental Health, Boston.*

24. Duration of action of anticholinesterases in dogs. *Alexander G. Karczmar (introduced by Theodore 'Koppanyi). Dept. Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*

25. Reversible blocking action of DFP at the neuromuscular junction of the rat diaphragm preparation. *Charles J. Kensler and Charles Berry (introduced by McKeen Cattell). Depts. Pharmacology and Anatomy, Cornell Univ. Medical College, New York City.*

26. Pharmacological actions of two aliphatic amines. *Theodore O. King (introduced by Theodore Koppanyi). Dept. Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*

27. Cholinesterase as a cholinergic blocking agent. *Theodore Koppanyi. Dept. Pharmacology, Georgetown Univ. School of Medicine, Washington, D. C.*

28. Species variation in the protective action of anesthesia against the acutely lethal effect of nicotine. *P. S. Larson, J. K. Finnegan, J. Conway Bibb* and H. B. Haag. Dept. Pharmacology, Medical College of Virginia, Richmond.*

29. A practical classification system for drugs. *C. D. Leake. Pharmacology Laboratory, Univ. of Texas Medical Branch, Galveston.*

30. Sodium phthalyl sulfacetimide—a new gastrointestinal antiseptic. *David Lehr. Dept. Pharmacology, New York Medical College, Flower and Fifth Avenue Hospitals, New York City.*

31. Comparative toxicities of five substituted glycerol ethers. *Prudence C. Loeb (introduced by Charles H. Hine). Division Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco.*

32. Comparison between individual sensitivities to histamine and to diphenhydramine. *S. Loewe, L. A. Woodbury* and Susanne Loewe Puttuck*. Depts. Pharmacology and Physiology, Univ. of Utah School of Medicine, Salt Lake City.*

33. Circulatory and other actions of sparteine in mammals. *Go Lu (introduced by P. J. Hanzlik). Dept. Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco.*

35. Acute and subacute toxicity of 1, 2, 3-trichloropropane in mice and rabbits. *Wm. A. McOmie and T. R. Barnes (introduced by Hamilton H. Anderson). Division Pharmacology and Exptl. Therapeutics, Univ. of California Medical School, San Francisco.*

36. A new antiadrenergic agent, 2-(N-p'-tolyl-N-(m'-hydroxyphenyl)-aminomethyl)-imidazoline HCl (C-7337). *Rolf Meier* and Frederick F. Yonkman. Research Depts. Ciba, Basle, Switzerland and Ciba, Summit, N. J.*

37. Mechanism of the acute lethal action of epinephrine in rats. *Mark Nickerson, John Berghout* and Robert N. Hammerstrom*. Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

38. Effects of pituitrin, pitressin and pitocin on blood pressure and cardiac automaticity as modified by various anesthetic agents. *Carlos E. Parsloe*, Lucien E. Morris* and O. Sidney Orth. Dept. Anesthesiology and Pharmacology, Univ. of Wisconsin Medical School, Madison.*

39. Quantitative estimation of pyribenzamine in blood. *Albert J. Plummer (introduced by George L. Maison). Dept. Pharmacology, Boston Univ. School of Medicine.*

40. Augmentor effect as postulated by the electromotive theory of drug action. *Harry J. Pratt* and R. Beutner. Hahnemann Medical College, Philadelphia.*

41. Lethal and electrocardiographic changes produced by quinine dihydrochloride in monkeys infected with P. knowlesi. *R. H. Rigdon (introduced by Chauncey D. Leake). Laboratory of Exptl. Pathology and Heart Station, Univ. of Texas Medical Branch, Galveston.*
42. Thiomerin: toxicity and diuretic effects. *A. Ruskin, J. E. Johnson, W. N. Roddy (introduced by Chauncey D. Leake). Heart Station, Univ. of Texas Medical Branch, Galveston.*
43. Further observations on isopropyl alcohol as anticonvulsant. *Ralph W. Schaffarzick* and P. J. Hanzlik. Dept. Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco.*
44. Production of ventricular extrasystoles in the dog by a polypropylene glycol. *F. E. Shideman and G. K. Moe. Dept. Pharmacology, Univ. of Michigan, Ann Arbor.*
45. Thiocyanate excretion. *Jesselene Thomas (introduced by Mark Nickerson). Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*
47. Thiouracil administration and thyroidectomy on experimental polyarthritis of rats. *Helen B. Tripi, William C. Kuzell and Grace M. Gardner (introduced by P. J. Hanzlik). Dept. Pharmacology and Therapeutics, Stanford Univ. School of Medicine, San Francisco.*
48. Algesimetry in the study of procaine anesthesia. *Paul E. Tullar (introduced by Theodore Koppanyi). Dept. Pharmacology, Georgetown Univ. School of Medicine, Washington, D.C.*
49. Effect of various lactones and of veratrine on the mammalian heart. *R. P. Walton, M. deV. Cotten* and O. J. Brodie*. Dept. Pharmacology, Medical College of South Carolina, Charleston.*
50. Effect of the isomers of methadon and isomethadon on the respiration of brain homogenates. *Daniel T. Watts (introduced by C. L. Gemmill). Dept. Pharmacology, Univ. of Virginia Medical School, Charlottesville.*
51. Studies on fate of certain antihistamines. *E. Leong Way, Robert E. Dailey* and Donald L. Howie*. Dept. Pharmacology, George Washington Univ. School of Medicine, Washington, D.C.*

THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Thirty-fourth Annual Meeting

An asterisk following an author's name denotes "by invitation"

TUESDAY, APRIL 19, 9:00 A.M.

Injury

LODGE ROOM C

1. Effect of renal ischemia on production of Nephrosis in jaundiced rabbits. *W. B. Wartman, J. M. Tucker*, A. P. Rusterholz*, and R. Jennings*. Dept. of Pathology, Northwestern Univ. Medical School, Chicago.*
2. Experimental papillary necrosis of the kidney. *Emanuel E. Mandel* and Hans Popper. Dept. of Pathology, Northwestern Univ. Medical School, the Hektoen Institute for Medical Research of the Cook County Hospital, and the U. S. Public Health Service, Chicago.*
3. Comparison of different lots of bacitracin for nephrotoxicity to rats and mice. *A. A. Nelson and E. C. Hagan*. Div. of Pharmacology, Food and Drug Adm., Federal Security Agency, Washington, D. C.*
4. Renal cytoplasmic inclusions following administration of large doses of streptomycin. *Charles W. Mushett and Robert B. Stebbins*. Merck Institute for Therapeutic Research, Rahway, N. J.*
5. Kidney protein associated with systemic arteriolar necrosis. *G. I. DeSuto-Nagy and L. L. Waters (introduced by M. C. Winternitz), Dept. of Pathology, Yale Univ. School of Medicine, New Haven.*
6. Cardiac hypertrophy. An immediate response to Starling's law of increased energy output of the heart. *Cecil A. Krakower and Helen E. Heino*. Univ. of Illinois College of Medicine, Chicago.*
7. Relations between volumes of closed hypothermal cerebral lesions and symptoms in rabbits. *C. Bruce Taylor*. George M. Hass, and John E. Maloney*. The Rush Dept. Pathology, Presbyterian Hospital, in affiliation with the Dept. Pathology, Univ. of Illinois College of Medicine, Chicago.*
8. Injury and recovery of mouse testes following whole-body exposure to x-radiation. *Allen Eschenbrenner. National Cancer Institute, Bethesda, Md.*
9. Cutaneous melanin production in the mouse by 5, 9, 10-trimethyl, 1-2 benzanthracene. *F. H. Burgoyne*, W. E. Heston*, J. L. Hartwell*, and H. L. Stewart. National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.*
10. Effect of antihistamine on localization of trypan blue in xylene-treated areas of skin. *R. H. Rigdon. Laboratory of Expt. Pathology, Medical Branch, Univ. of Texas, Galveston.*
11. Spontaneous adenomatous gastritis of mice of strain dba. *William V. Hare* and Harold L. Stewart. National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.*
12. Response of the rachitic metaphysis in the rat to excessive administration of vitamin A. *S. B. Wolbach and Charlotte L. Maddock*. Division of Nutritional Research, The Children's Hospital, Boston.*

TUESDAY, APRIL 19, 9:00 A.M.

Infection

ROOM 3

1. Intradermal reaction to streptococcal thermal antibody and antigen in persons having epidemic respiratory infections. *Edward C. Rosenow. Bacteriological Research, Longview Hospital, Cincinnati.*
2. Electron microscope studies on virus-cell relationships. *F. B. Bang and G. O. Gey*. Dept. Medicine and Surgery, Johns Hopkins Medical School, Baltimore.*
3. Effect of malnutrition on infection of mice with influenza virus. *Douglas H. Sprunt. Div. Pathology and Bacteriology, Univ. of Tennessee College of Medicine, Memphis.*
4. Intradermal vaccination with infectious psittacosis virus. Virus distribution. *John C. Wagner* and Joseph Victor. Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.*
5. Intradermal vaccination with infectious psittacosis virus. Pathological changes. *Joseph Victor and John C. Wagner*. Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.*
6. Thermostable inhibition of bacterial hyaluronidases by serum of normal human beings. *Robert T. Thompson and Frances E. Moses (introduced by M. A. Blankenhorn). Dept. of*

Internal Medicine, College of Medicine, Univ. of Cincinnati.

7. Bactericidal action of dog blood against *Br. Suis*. *E. Buist Wells*, Jean Clements*, Abou D. Pollack*, and Joseph Victor. Biological Dept., Chemical Corps, Camp Detrick, Frederick, Md.*
8. Prophylaxis of allergic acute disseminated encephalomyelitis in guinea pigs with salicylate and para-aminobenzoic acid. *Robert A. Good, Berry Campbell, and T. A. Good (introduced by Irvine McQuarrie). Univ. of Minnesota, Minneapolis.*
9. Passive transfer of age resistance to an avian malaria parasite. *William Trager and R. Barclay McGhee*. Dept. Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.*
10. Electrocardiograms of normal and malarial infected monkeys. *Arthur Ruskin* and R. H. Rigdon. Heart Station and Laboratory of Exptl. Pathology, Univ. of Texas, Medical Branch, Galveston.*
11. Contagiousness of coccidioidomycosis. *Sol Roy Rosenthal. Univ. of Illinois, College of Medicine, Chicago.*
12. Varying genetic resistance of rabbits to quantitative inhalation of human tubercle bacilli. *Max B. Lurie, Samuel Abramson*, and A. G. Heppleston*. Henry Phipps Institute, Univ. of Pennsylvania, Philadelphia.*

TUESDAY, APRIL 19, 1:30 P.M.

Blood

LODGE ROOM C

1. Iron excretion in human subjects as measured by isotope technique. *Reubenia Dubach*, Carl V. Moore, and Sheila Callender*. Washington Univ. School of Medicine, St. Louis.*
2. Intravenous administration of commercial cytochrome to rats previously treated with radioactive iron. *Helmut Beinert and Kurt R. Reissman (introduced by Fred W. Oberst). USAF School of Aviation Medicine, Randolph Air Force Base, Texas (Biochem.).*
3. Mechanical and osmotic fragility in relation to age of dog erythrocytes tagged with radioiron. *W. B. Stewart, J. M. Stewart, M. J. Izzo, and L. E. Young (introduced by C. L. Yuile). Depts. Pathology and Medicine, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
4. Reticulocyte ripening and nutrition in anemic dogs. *A. Nizet (ingroduced by F. S. Robscheit-Robbins). Dept. Pathology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
5. Production of 'sickling' in normal red blood cells. *Raphael Isaacs. Chicago.*
6. Blood dyscrasia in mice ingesting toluidine blue. *Thelma B. Dunn. National Cancer Institute, Bethesda, Md.*
7. Experimental production of a nutritional macrocytic anemia in swine. *G. E. Cartwright* and M. M. Wintrobe. Dept. Medicine, Univ. of Utah College of Medicine, Salt Lake City.*
8. Actions of 4-amino-pteroylglutamic acid in dogs with special reference to hematopoiesis. *J. B. Thiersch and F. S. Philips (introduced by C. P. Rhoads). Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.*
9. Determination of blood iodine, a simplified method for use in the clinical laboratory. *Arthur C. Connor*, Roy E. Swenson*, and George M. Curtis. Dept. of Research Surgery, Ohio State Univ., Columbus.*
10. Leukocyte production and distribution: factors in plasma and in leukocytes. *Bernhard Steinberg and Ruth A. Martin*. Toledo Hospital Institute of Medical Research.*
11. Nitrogen mustards as effective blood and plasma sterilizing agents. *Frank W. Hartman, and George H. Mangun*. Dept. of Laboratories, Henry Ford Hospital, Detroit.*
12. Electron microscopy of clinical marrow aspirates. *J. W. Rebuck and H. L. Woods (introduced by F. W. Hartman). Dept. of Laboratories, Henry Ford Hospital, Detroit.*

TUESDAY, APRIL 19, 1:30 P.M.

Inflammation; Deficiencies; Miscellaneous

ROOM 3

1. Inorganic composition and phosphatase activity of human cartilage in relation to morphological differentiation. *Richard H. Follis, Jr. Dept. Pathology, Johns Hopkins Univ., Baltimore.*
2. Preparation and properties of mammalian striated myofibrils. *Armin F. Schick* and George M. Hass. The Rush Dept. Pathology, Presbyterian Hospital, Chicago, in affiliation with the Dept. Pathology, Univ. of Illinois College of Medicine, Chicago.*
3. Effect of pH on the cultivation of macrophages *in vitro*. *I. N. Dubin and C. K. Yen*. Division Pathology and Bacteriology, Univ. of Tennessee College of Medicine, Memphis.*
4. A macrophage promoting factor (MPF) in mammalian blood serum. *C. M. Pomerat and M. F. Orr*. Tissue Culture Laboratory, Medi-*

cal Branch, Univ. of Texas, Galveston (Physiol.).

5. Mercury content of human tissues from routine autopsy material. *Robert B. Forney (introduced by H. R. Hulpieu) and R. N. Harger. Dept. Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis (Pharmacol.).*
6. Vitamin B_6 deficiency in the syrian hamster. *Gregory Shwartzman and Lotte Strauss*. Div. of Bacteriology, The Laboratories of The Mount Sinai Hospital, New York City.*
7. Pathologic changes in guinea pigs produced by diets deficient in anti-stiffness factor. *Paul N. Harris and Rosalind M. Wulsen*. The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, and Dept. Zoology, Oregon State College, Corvallis (Pharmacol.).*
8. Localization of vital dyes in areas of inflammation. *Howard C. Hopps and Barbara C. Reiter*. Dept. Pathology, Univ. of Oklahoma School of Medicine, Oklahoma City.*
9. Pathogenesis of experimental necrotizing arteritis. *J. H. McCormick, Jr.*, and Russell L. Holman. Dept. Pathology, Louisiana State Univ. School of Medicine, New Orleans.*
10. Color reactions with malignant sera. *Emil Weiss. Tumor Clinic, Michael Reese Hospital, Chicago (Immunol.).*
11. An additional thermostable component in the mechanism of leukocytosis with inflammation. *Valy Menkin. Agnes Barr Chase Foundation for Cancer Research, Temple Univ. School of Medicine, Philadelphia.*

TUESDAY, APRIL 19, 8:00 P.M.

Federation Joint Session

AUDITORIUM

Program on p. 418

WEDNESDAY, APRIL 20, 9:00 A.M.

Atomic Energy

LODGE ROOM C

Shields Warren, *Chairman*

1. Radioresistance and radiosensitivity of swine exposed to atomic bomb radiation. *John L. Tullis. Commander, MC, U. S. Navy, National Medical Center, Bethesda, Md.*
2. Body content of radium in individuals with no known exposure. *J. B. Hursh and A. A. Gates. Dept. Radiation Biology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*
3. Radiation effects on tissues studied with C^{14}-Labeled glycine. *Kurt I. Altman, George W. Casarett, Thomas R. Noonan, and Kurt Salomon. Dept. Radiation Biology, Univ. of Rochester, Rochester, N. Y.*
4. Absorption and excretion of carbon^{14} as CO_2 and bicarbonate. *A. M. Brues and Agnes Naranjo. Argonne National Laboratory, Chicago.*
5. Effect of acute x-radiation on distribution and excretion of radio sodium in the rat. *L. R. Bennett, Vera C. Bennett, and Joe W. Howland. Dept. Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
6. Bone deposition of uranium. *W. F. Neuman, M. W. Neuman, E. R. Main, and B. J. Mulryan. Dept. Radiation Biology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*
7. Lung pathology following the inhalation of radioactive cerium. *Hermann Lisco and M. P. Finkel. Argonne National Laboratory, Chicago.*
8. Effects of x-rays on the metabolism of nucleic acids in hematopoietic tissues. *Charles E. Carter. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.*
9. Cytological effects of ionizing radiations—their subsequent lethality. *A. D. Conger. Oak Ridge National Laboratory, Oak Ridge, Tennessee.*
10. Influence of a conditioning injection of estrogen on hematologic and organ weight response to x-irradiation. *H. M. Patt, Marguerite N. Swift, Robert L. Straube, Ella B. Tyree, and Douglas E. Smith. Argonne National Laboratory, Chicago.*
11. X-ray induced developmental abnormalities in the mouse. *Liane Brauch Russell. Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.*
12. Pathology of an experimental flash-burn. *Lewis Hogg, J. Thomas Payne, and Herman E. Pearse. Univ. of Rochester Atomic Energy Commission Project, Rochester, N. Y.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Blood Coagulation

LODGE ROOM C

Joint Session with the American Society of Biological Chemists

1. Canine hemophilia: the clotting anomaly and the effectiveness of transfusions. *John B.*

Graham, Joseph A. Buckwalter*, L. J. Hartley* and K. M. Brinkhous. Dept. Pathology, Univ. of North Carolina, Chapel Hill.*

2. Hemostatic effects of normal blood transfusions in hemophilia-like swine. *M. E. Muhrer (introduced by A. G. Hogan). Dept. Agricultural Chemistry and Animal Husbandry, Univ. of Missouri, Columbia (Biochem.).*
3. Effect of various adsorbants on the components of the prothrombin complex. *Mario Stefanini* and Armand J. Quick. Dept. Biochemistry, Marquette Univ. School of Medicine, Milwaukee (Biochem.).*
4. Activation of purified prothrombin with sodium citrate. *Walter H. Seegers and Arnold G. Ware*. Dept. Physiology and Pharmacology, Wayne Univ., Detroit (Biochem.).*
5. Effect of lecithin in the formation of thrombin. *Joseph E. Flynn and Eugene T. Standley (introduced by H. P. Smith). Dept. Pathology, Columbia Univ., New York City.*
6. The prothrombin consumption test. *Armand J. Quick and Jean Favre-Gilly*, Dept. Biochemistry, Marquette Univ. School of Medicine, Milwaukee (Biochem.).*
7. Hypoprothrombinemia due to loss of intestinal lymph. *J. D. Mann*, F. D. Mann*, and J. L. Bollman. Mayo Foundation, Rochester, Minn.*
8. Antithromboplastic activity of navy bean trypsin inhibitor in vivo. *Donald E. Bowman. Dept. Biochemistry and Pharmacology, Indiana Univ. School of Medicine, Indianapolis (Biochem.).*
9. In-vitro histamine release from blood cells of sensitized rabbits—relationship to blood-coagulation mechanisms. *L. W. Roth, Floyd C. McIntire* and R. K. Richards. Dept. of Pharmacology and Biochemistry, Abbott Research Laboratories, North Chicago (Physiol.).*
10. Preparation and preservation of fraction I from human plasma. *John T. Edsall, John D. Ferry, and Peter R. Morrison*. Dept. Physical Chemistry, Harvard Medical School, Boston (Biochem.).*
11. Effect of methyl-bis(beta-chloroethyl)amine on blood constituents. *George H. Mangun. Dept. of Laboratories, Henry Ford Hospital, Detroit (Biochem.).*

WEDNESDAY, APRIL 20, 9:30 P.M.

Federation "Mixer"

HOTEL STATLER BALLROOM

THURSDAY, APRIL 21, 9:00 A.M.

Liver—Tumors

LODGE ROOM C

1. Histopathology of the liver following histamine infusion. *W. G. Nothacker* and Ralph W. Brauer (introduced by Russell L. Holman). Depts. Pathology and Pharmacology, Louisiana State Univ., School of Medicine, New Orleans.*
2. Clearance of rose bengal by livers of normal and carbon tetrachloride-treated mice. *W. Lane Williams. Dept. Anatomy, Univ. of Minnesota, Minneapolis.*
3. Effects of 2-acetylaminofluorene on liver function. *James B. Allison, Arthur W. Wase*, and Walter W. Wainio*. Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J. (Biochem.).*
4. Quantitative correlation between results of hepatic tests and morphologic liver cell damage. *Hans Popper, Frederick Steigmann, and Paul B. Szanto*. Hektoen Institute for Medical Research of the Cook Country Hospital, Dept. Pathology of Northwestern Univ. Medical School, and the Dept. Internal Medicine, Univ. of Illinois College of Medicine, Chicago.*
5. Massive hepatic necrosis in the protein-depleted, partially hepatectomized rat fed hypertonic diets. *Robert L. Estrada*, Zachary A. Simpson*, Harry M. Vars*, and I. S. Ravdin. Harrison Dept. Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*
6. Prompt and indirect reacting serum bilirubin in jaundice. *Fenton Schaffner*, Hans Popper, and Frederick Steigmann. Hektoen Institute for Medical Research of the Cook County Hospital, Dept. Pathology of Northwestern Univ. Medical School, and the Dept. Internal Medicine, Univ. of Illinois College of Medicine, Chicago.*
7. Methionine and choline in arrest of dietary cirrhosis of the liver in the rat. *Ernest R. Jaffe*, Robert W. Wissler* and Earl P. Benditt. Dept. Pathology, Univ. of Chicago, Chicago.*
8. Blood supply of neoplasms in the liver. *Charles Breedis and Gang Young*. Dept. Pathology, Medical School, Univ. of Pennsylvania, Philadelphia, and Pathology Research Institute, Medical College, National Sun Yat Sen Univ., Canton, China.*
9. Induction of hepatic carcinoma in rats by 4-dimethylaminobenzene - 1 - azo - 1 - naphthalene. *A. S. Mulay (introduced by Harold L. Stewart) and H. I. Firminger (introduced*

by Harold L. Stewart). National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Md.

10. Incidence of neoplasms and pathological changes in rats as a result of prolonged choline deficiency. *Cyrus C. Erickson and Walter Goebbel*. Dept. Pathology and Dept. Surgery, Duke Medical School, Durham, N. C.*
11. Lipids in the lymph of rats. *J. L. Bollman and E. V. Flock. Mayo Foundation, Rochester, Minnesota.*
12. Association of lymphoid elements with cancer cells undergoing distinctive necrobiosis in resistant and immune hosts. *Helene W. Toolan* and John G. Kidd. Dept. Pathology, The New York Hospital-Cornell Medical Center, New York City.*
13. Fate of cancer cells implanted in susceptible and resistant hosts. *John G. Kidd and Helene W. Toolan*. Dept. Pathology, The New York Hospital-Cornell Medical Center, New York City.*

THURSDAY, APRIL 21, 1:30 P.M.

Protein Metabolism

LODGE ROOM C

1. Nucleic acids and the production of antibody by plasma cells. *Carolyn Forman*, William E. Ehrich and David L. Drabkin. Depts. Pathology and Biochemistry, Graduate School of Medicine, Univ. of Pennsylvania, and the Philadelphia General Hospital.*
2. Effect of ageing on the protein fractions of the normal human heart. *John W. Harman and Jean H. Webster (introduced by D. Murray Angevine). Dept. Pathology, Univ. of Wisconsin Medical School, Madison.*
3. Electrophoretic findings in normal and abnormal pregnancy. *Nevin S. Scrimshaw* and Eric L. Alling. School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*
4. Level of serum mucoprotein as indicator of disease activity in rheumatic fever. *V. C. Kelley and R. A. Good (introduced by Irvine McQuarrie). Dept. Pediatrics, Univ. of Minnesota, Minneapolis.*
5. Vitamin deficiency and protein synthesis with special reference to riboflavin-protein interrelationships. *Robert W. Wissler*, Earl P. Benditt, C. Harold Steffee* and Robert L. Woolridge*. Dept. Pathology, Univ. of Chicago.*
6. Further studies on proteinuria in dogs. *Roger Terry (introduced by G. H. Whipple). Dept. Pathology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*
7. Fates of parenterally administered homologous serum protein and casein hydrolysate. *John Fuller*, E. M. Humphreys*, C. H. Steffee*, R. W. Wissler* and E. P. Benditt. Dept. Pathology, Univ. of Chicago.*
8. Determination of minimal daily essential amino acid requirements in protein-depleted male albino rats. *C. H. Steffee*, R. W. Wissler*, E. M. Humphreys*, E. P. Benditt, R. L. Woolridge* and P. R. Cannon. Dept. Pathology, Univ. of Chicago.*
9. Minimum daily requirements of indispensable amino acids for maintenance of adult rats. *L. E. Frazier*, R. L. Woolridge*, C. H. Steffee*, R. W. Wissler* and E. P. Benditt. Dept. Pathology, Univ. of Chicago.*
10. Cytochrome oxidase, succinoxidase and phosphatase activities of tissue of rats on protein-deficient diets. *E. P. Benditt, C. H. Steffee*, T. Hill* and T. L. Johnston*. Dept. Pathology, Univ. of Chicago.*
11. Effects of protein deficiency on natural and acquired immunity to Friedlander's bacillus. *Robert L. Woolridge (introduced by Paul R. Cannon). Dept. Pathology, Univ. of Chicago.*
12. Comparison of two types of experimental ascites in the dog. *Wade Volwiler*, Jesse L. Bollman and John H. Grindlay. Mayo Foundation, Rochester, Minn.*

THURSDAY, APRIL 21, 4:30 P.M.

Pathology Business Meeting

LODGE ROOM C

FRIDAY, APRIL 22, 9:00 A.M.

Endocrines—Cholesterol

LODGE ROOM C

1. Effect of thyroid feeding on histologically normal islets of rats receiving small doses of alloxan. *David W. Molander* and Arthur Kirschbaum. Dept. Anatomy, Univ. of Minnesota Medical School, Minneapolis.*
2. Retardation of the reaction time in parathyroidectomized rats. *Carl C. Smith* and Herbert C. Stoerk. Merck Institute for Therapeutic Research, Rahway, N. J.*
3. Parathormone and renal reabsorption of phosphate. *Herbert C. Stoerk and Robert H. Silber*. Merck Institute for Therapeutic Research, Rahway, N. J.*
4. Influence of chorionic gonadotrophin on hilus cells of the human ovary. *William H. Sternberg*, Albert Segaloff and Cornelia J. Gas-*

kill. Depts. Pathology and Medicine, Tulane Univ., Alton Ochaner Medical Foundation, and the Charity Hospital of Louisiana, New Orleans.*

5. Chorionic gonadotrophin as luteotrophin in women. *Albert Segaloff, Cornelia J. Gaskill*, William Sternberg* and Richard L. Coppedge.* Depts. Medicine, Physiology and Pathology, Tulane Univ., Alton Ochsner Medical Foundation, and the Charity Hospital of Louisiana, New Orleans.*
6. Eclampsia-like condition in pregnant rats injected with progesterone. *A. Symeonidis (introduced by H. L. Stewart). National Cancer Institute, Bethesda, Md.*
7. Multiple injections of potassium as a test for adrenocortical function, with modifications induced by desoxycorticosterone. *Theodore Robertson (introduced by John G. Kidd). Dept. Pathology, New York Hospital-Cornell Medical Center, New York City.*
8. Blood cholesterol value of normal men in relation to their basal metabolic rate. *Annie Laurie Peeler*, Opal E. Hepler, Velma Miller Kinney*, Lillian E. Cisler* and Frederic T. Jung*. Depts. Pathology and Physiology, Northwestern Univ. Medical School and Passavant Memorial Hospital, Chicago.*
9. Sustained elevation of blood cholesterol and phospholipid levels in rabbits given detergents intravenously. *Aaron Kellner, James W. Correll and Anthony T. Ladd (introduced by John G. Kidd). Dept. Pathology, New York Hospital-Cornell Medical Center, New York City.*
10. Intravenous detergents in experimental atherosclerosis, with special reference to the possible role of phospholipids. *Anthony T. Ladd, Aaron Kellner, and James W. Correll (introduced by John G. Kidd). Dept. Pathology, New York Hospital-Cornell Medical Center, New York City.*
11. Influence of alloxan diabetes on cholesterol atherosclerosis in the rabbit. *Henry C. McGill*, Louis E. Parrish* and Russell L. Holman. Dept. Pathology, Louisiana State Univ., School of Medicine, New Orleans.*
12. Cytological effects of alloxan in intact and thymectomized rats. *Thurlo B. Thomas*, Paul L. Ewing and G. A. Emerson. Dept. Zoological Sciences, Carleton College, Northfield, Minn., and Dept. Pharmacology, Univ. of Texas Medical Branch, Galveston (Pharm.).*

Papers Read by Title

1. Replacement of a portion of common bile duct by a segment of uterine horn. *Charles M. O'Leary*, William T. Snoddy* and Bela Halpert. Depts. Surgery and Pathology, Univ. of Oklahoma School of Medicine, Oklahoma City.*
2. Influence of nutrition on plasma vitamin A alcohol. *Hans Popper, Frederick Steigmann, Hattie A. Dyniewicz* and Irene A. Maxwell*. Hektoen Institute for Medical Research of the Cook County Hospital, Dept. Pathology of Northwestern Univ. Medical School, and Dept. Internal Medicine, Univ. of Illinois College of Medicine, Chicago.*

AMERICAN INSTITUTE OF NUTRITION

Thirteenth Annual Meeting

An asterisk following an author's name denotes "by invitation"

TUESDAY, APRIL 19, 9:00 A.M.

Amino Acids

LODGE ROOM B

1. Effect of various amino acids on rats receiving niacin-tryptophan deficient rations. *L. V. Hankes, L. M. Henderson* and C. A. Elvehjem. Dept. Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.*
2. Role of the gastrointestinal tract in the conversion of tryptophan to nicotinic acid. *James M. Hundley (introduced by F. S. Daft). Exptl. Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.*
3. Tryptophan-nicotinic acid relationship in the developing chick embryo. *Charles A. Denton*, W. L. Kellogg*, W. E. Rowland* and H. R. Bird. Bureau of Animal Industry, Agricultural Res. Adm., U. S. D. A., Beltsville, Md.*
4. Effect of vitamin nutriture on tryptophan metabolism in man. *Herbert P. Sarett and Grace A. Goldsmith. Nutrition Research Laboratory, Tulane Univ. School of Medicine, New Orleans.*
5. Leucine content and utilization of amino acid mixtures. *Joseph T. Anderson* and E. S. Nasset. Dept. Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*
6. The concurrent feeding of amino acids. *Robert S. Harris and Richard Henderson* Nutritional Biochemistry Laboratories, Mass. Inst. of Technology, Cambridge, Mass.*
7. Rat-repletion studies with amino acid solutions. Role of non-essential amino acids. *Douglas V. Frost and Harry R. Sandy*. Nutritional Research Division, Abbott Laboratories, North Chicago.*
8. Lysine requirement for growing pigs. *M. J. Brinegar*, J. K. Loosli, Harold H. Williams and L. A. Maynard. Depts. Animal Husbandry and Biochemistry and Nutrition, Cornell Univ., Ithaca.*
9. Synthesis of amino acids in the rumen. *W. E. Thomas*, J. K. Loosli, Fent H. Ferris*, Harold H. Williams and L. A. Maynard. Depts. of Animal Husbandry and Biochemistry and Nutrition, Cornell Univ., Ithaca.*
10. Role of methionine in the protein metabolism of the rat. *Cecile Hoover*, Pearl Swanson and Helen Merriam Stewart*. The Nutrition Laboratory, the Foods and Nutrition Section, Iowa Agricultural Experiment Station, Iowa State College, Ames.*
11. Tyrosine excretion in rheumatoid arthritics and normals. *E. B. Wallraff*, C. A. Stephens*, A. Borden*, P. Holbrook*, D. Hill*, L. J. Kent* and A. R. Kemmerer. Dept. Nutrition, Univ. of Arizona, and Southwestern Clinic and Research Institute, Tucson.*
12. Blood changes in acute amino acid deficiencies. *W. Knowlton Hall, Lester L. Bowles* and V. P. Sydenstricker*. Depts. Biochemistry, Microscopic Anatomy and Medicine, Univ. of Georgia School of Medicine, Augusta (Biochem.).*

TUESDAY, APRIL 19, 1:30 P.M.

Proteins

LODGE ROOM B

1. Influence of dietary proteins on the urinary excretion of amino acids by human subjects. *Betty F. Steele*, May S. Reynolds* and C. A. Baumann. Dept. Biochemistry and Home Economics, College of Agriculture, Univ. of Wisconsin, Madison.*
2. Growth and reproduction of rats on rations devoid of animal protein. *M. O. Schultze (introduced by G. M. Briggs). Division Agricultural Biochemistry, Univ. of Minnesota, Minneapolis.*
3. The effect of carbohydrate on the nutritive value of heated protein. *Ivan J. Mader*, Lawrence J. Schroeder* and Arthur H. Smith. Dept. Physiological Chemistry, Wayne Univ. College of Medicine, Detroit.*
4. Studies involving protein stores in man. *Stanley Levey, John E. Harroun and Charley J. Smyth (introduced by Arthur H. Smith). Wayne County General Hospital, Eloise, Michigan, and the Depts. Physiological Chemistry and Medicine, Wayne Univ. College of Medicine, Detroit.*
5. Nitrogen excretion of women related to the distribution of animal protein in the daily meals. *Ruth M. Leverton and Mary R. Gram*. Human Nutrition Research Laboratory, Univ. of Nebraska, Lincoln.*

6. Influences of dietary protein. I. Transmittal of growth factors. *Frank A. Csonka and M. W. Olsen*. Bureau of Human Nutrition and Home Economics and the Bureau of Animal Industry, Agricultural Res. Adm. U. S. D. A., Washington, D. C.* (*Biochem.*).
7. Evaluation of six standard proteins by growth of young dogs. *Dorothea Mabee* and Agnes Fay Morgan. Laboratory Home Economics, Univ. of California, Berkeley.* (*Biochem.*).
8. Utilization of human serum albumin injected intravenously. *Cecilia Riegel and Nicholas Gimbel*. Harrison Dept. Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia* (*Biochem.*).
10. Sulfur-free protein hydrolysates for cystine- and methionine-free diets. *John L. Wood, Shirley Lucille Cooley* and Nelson Kingsland*. Dept. Chemistry, School of Biological Sciences, Univ. of Tennessee, Memphis* (*Biochem.*).
11. Microbially synthesized APF (animal protein factor). *Stefan Ansbacher, Harley H. Hill, Jr.*, Jesse W. Tieman*, Jean F. Downing* and John H. Caldwell, Jr.* Research Laboratory, Vi-D-Co. Marion, Ind.* (*Biochem.*).

TUESDAY, APRIL 19, 4:30 P.M.

Business Meeting

LODGE ROOM B

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

WEDNESDAY, APRIL 20, 9:00 A.M.

Vitamins A, D, E; Thiamine; Pyridoxine

LODGE ROOM B

1. A paradoxical relationship between serum level and liver content of vitamin A. *R. F. Krause* (*introduced by H. B. Pierce*). *Dept. Biochemistry, College of Medicine, Univ. of Vermont, Burlington.*
2. Vitamin A requirement of the turkey poult fed a purified diet. *Walter C. Russell, M. Wight Taylor and Robert Van Reen*. Dept. Agricultural Biochemistry, New Jersey Agricultural Experiment Station, Rutgers Univ., New Brunswick.*
3. Chronic vitamin A deficiency in three inbred strains of mice. *Paul F. Fenton, Ruth E. Shrader* and George R. Cowgill. Yale Nutrition Laboratory, Dept. Physiological Chemistry and Dept. Anatomy, Yale Univ., New Haven.*
4. Fasting serum vitamin A and carotene levels and vitamin A absorption in males over 40. *Marvin J. Yiengst* and Nathan W. Shock. Section on Cardiovascular Diseases and Gerontology, National Heart Institute, National Institutes of Health, and Baltimore City Hospitals, Baltimore* (*Physiol.*).
5. Studies with aqueous dispersions of fat-soluble vitamins. *Albert E. Sobel, Abraham Rosenberg*, Roy Geduldig*, Eli Engel*, Michael West* and Benjamin Kramer. Dept. Chemistry and Pediatrics, Jewish Hospital of Brooklyn* (*Biochem.*).
6. Determination of vitamin D activity in the rat by means of radioactive phosphorus. *R. H. Snyder*, Harold J. Eisner*, and Harry Steenbock. Dept. Biochemistry, Univ. of Wisconsin, Madison.*
7. Fatal lung hemorrhage and liver damage in rats prevented by vitamin E. *E. L. Hove, D. H. Copeland* and W. D. Salmon. Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn.*
8. Some inter-relationships of thiamine and fat in the nutrition of the rat. *M. M. Krider* and N. B. Guerrant. Dept. Agricultural and Biological Chemistry, The Pennsylvania State College, State College.*
9. Decrease in the storage of thiamine in tissues from supplementing the diet with live bakers' yeast. *Fung-Haan Fung*, Ruth J. Aaness* and Helen T. Parsons. Dept. Home Economics, Univ. of Wisconsin, Madison.*
10. Desoxypyridoxine and development of the chick embryo. *W. W. Cravens and E. E. Snell. Dept. Poultry Husbandry and Biochemistry, Univ. of Wisconsin, Madison.*
11. Human urinary and fecal elimination of vitamin B_6 and 4-pyridoxic acid on a controlled diet. *Hellen Linkswiler and May S. Reynolds* (*introduced by Helen T. Parsons*). *Dept. Home Economics, Univ. of Wisconsin, Madison.*
12. Role of pyridoxine in economy of food utilization. *Barnett Sure and Leslie Easterling*. Univ. of Arkansas, Fayetteville.*

WEDNESDAY, APRIL 20, 1:30 P.M.

Pteroylglutamic Acid; Vitamin B_{12}; Choline; Biotin

LODGE ROOM B

1. Folic acid deficiency and the respiration and pyruvate utilization of duckling erythrocytes and cardiac muscle. *O. Neal Miller*, Robert E. Olson* and Frederick J. Stare. Dept. Nutrition, Harvard School of Public Health and Dept. Biological Chemistry, Harvard Medical School, Boston.*
2. Relation of factor in a methanol extract of liver and pteroylglutamic acid in mink. *S. B. Tove* and C. A. Elvehjem. Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.*
3. Effects of pteroylglutamic acid, injectable liver extract, and ascorbic acid on dietary glycine toxicity. *James S. Dinning*, Cecilia K. Keith*, John R. Totter* and Paul L. Day. Dept. Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock.*
4. Pteroylglutamic acid studies on patients with tropical sprue: factors affecting urinary excretion levels. *Elizabeth M. Knott and Ramón M. Suárez (introduced by R. R. Williams). The School of Tropical Medicine, San Juan, Puerto Rico.*
5. Vitamin B_{12}—a growth factor for young rats. *Gladys A. Emerson, Elizabeth Wurtz* and Mary E. Zanetti*. Merck Institute for Therapeutic Research, Rahway, N. J.*
6. Vitamin B_{12} in swine nutrition. *A. G. Hogan and G. C. Anderson*. Depts. Animal Husbandry and Agricultural Chemistry, Univ. of Missouri, Columbia.*
7. Essentiality of vitamin B_{12} for the baby pig, with preliminary quantitative data. *A. L. Neumann*, Marian F. James*, J. L. Krider* and B. Connor Johnson. Dept. Animal Science and Division of Animal Nutrition, Univ. of Illinois.*
8. Animal protein factor and growth of mice fed diets containing succinylsulfathiazole. *D. K. Bosshardt, J. W. Huff*, Winifred J. Paul*, and R. H. Barnes. Dept. Biochemistry, Medical Research Division, Sharp and Dohme, Inc., Glenolden.*
9. Relation of vitamin B_{12} to the choline requirement of the rat and chick. *A. E. Schaefer*, W. D. Salmon and D. R. Strength*. Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn.*
10. Amino acids and vitamin B_{12} as supplements to plant proteins for growing chicks. *L. R. Richardson, L. G. Blaylock*, H. L. German* and R. M. Sherwood*. Texas Agricultural Experiment Station, College Station.*
11. Relation of choline intake to survival in rats fed p-dimethylaminoazobenzene. *R. W. Engel. Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn.*
12. Effect of biotin deficiency in ducklings upon the histopathology of the heart. *James W. Goddard and Robert E. Olson (introduced by Frederick J. Stare). Dept. Nutrition, Harvard School of Public Health, Boston.*

WEDNESDAY, APRIL 20, 7:00 P.M.

American Institute of Nutrition Dinner
Presentation of Awards

BOOK-CADILLAC HOTEL, CRYSTAL BALLROOM

WEDNESDAY, APRIL 20, 9:30 P.M.

Federation Mixer

HOTEL STATLER BALLROOM

THURSDAY, APRIL 21, 9:00 A.M.

Vitamin C; Rehabilitation

LODGE ROOM B

1. Excretion of ascorbic acid on supplements of cantaloupe and crystalline ascorbic acid. *Shih Dzung Chen* and Cecilia Shuck. Nutrition Laboratory, School of Home Economics, Purdue Univ., Lafayette, Ind.*
2. Relation of age to ascorbic acid metabolism in guinea pigs. *Mary Elizabeth Reid. Exptl. Biol. and Med. Institute, National Institutes of Health, Bethesda, Md.*
3. Vitamin C deficiency in the ringtail monkey. *James H. Shaw. School of Dental Medicine, Harvard Univ., Boston.*
4. A study of ascorbic acid metabolism of older adolescents. *Clara A. Storvick, Bessie L. Davey*, Ruth M. Nitchals*, Ruth E. Coffey* and Margaret L. Fincke. Nutrition Research Laboratory, Agricultural Experiment Station, Oregon State College, Corvallis.*
5. Studies on the fate of ascorbic acid, dehydroascorbic acid and diketogulonic acid in the animal body. *Mary B. Mills*, Charlotte M. Damron*, and Joseph H. Roe. Dept. Biochemistry, George Washington Univ., Washington, D. C.*
6. Ascorbic acid metabolism in patients with certain gingival disturbances. *Raymond Hayes*, Walter M. Booker and Marianna B.*

Sewell. Dept. of Oral Medicine, College of Dentistry, and Dept. Pharmacology, College of Medicine, Howard Univ., Washington, D. C. (Physiol.).

7. Weight of liver and liver glycogen of rats after dietary restriction and rehabilitation. *Ruth M. Clayton and Esther Da Costa (introduced by Robert E. Johnson). U. S. Army Medical Nutrition Laboratory, Chicago.*
8. Effect of dietary restriction and rehabilitation on serum protein of male rats. *Esther Da Costa and Ruth Clayton (introduced by Robert E. Johnson). U. S. Army Medical Nutrition Laboratory, Chicago.*
9. Effect of dietary restriction and rehabilitation upon the spontaneous activity of rats. *Harry Krzywicki and Esther Da Costa (introduced by Robert E. Johnson). U. S. Army Medical Nutrition Laboratory, Chicago.*
10. Nutritional requirements of men living under survival conditions in cold weather. *C. G. Bly*, R. E. Johnson and C. F. Consolazio*. U. S. Army Medical Nutrition Laboratory, Chicago.*
11. Effect of diet composition on rehabilitation following starvation in albino rats. *W. A. Krehl and W. P. McNulty, Jr.* Yale Nutrition Laboratory, Dept. of Physiological Chemistry, Yale Univ., New Haven (Biochem.).*
12. Resurvey of nutritional status in Norris Point, Newfoundland. *Grace A. Goldsmith, William J. Darby, Ruth Steinkamp*, Anne Stockell* and Ellen McDevitt*. Dept. Medicine, Tulane Univ. School of Medicine, New Orleans, Depts. Biochemistry and Medicine, Vanderbilt Univ. School of Medicine, Nashville.*

THURSDAY, APRIL 21, 1:30 P.M.

Riboflavin; Pantothenic Acid; Metabolism; Lipids

LODGE ROOM B

1. Riboflavin and nitrogen metabolism of six women with active tuberculosis. *Wilma D. Brewer*, Helen L. Tobey* and Margaret A. Ohlson. Dept. Foods and Nutrition, School of Home Economics, Michigan State College, East Lansing.*
2. Relationship of riboflavin deficiency to adrenicortical function as shown by carbohydrate metabolism in anoxia. *Bluebell Reade* and Agnes Fay Morgan. Laboratory of Home Economics, Univ. of California, Berkeley.*
3. Metabolism of radioactive acetate and pyruvate by cardiac muscle from normal and pantothenic acid-deficient ducklings. *Robert E. Olson* and Fredrick J. Stare. Dept. Nutrition, Harvard School of Public Health and Dept. Biological Chemistry, Harvard Medical School, Boston.*
4. Availability of panthenol and calcium pantothenate in relation to food intake. *S. H. Rubin, L. Drekter*, M. E. Moore* and R. Pankopf*. Nutrition Laboratories, Hoffmann-LaRoche Inc., Nutley, N. J.*
5. Metabolism of p-aminobenzoic acid and some of its conjugates by the rat. *Thomas R. Riggs* and D. Mark Hegsted. Dept. Nutrition, Harvard School of Public Health, Dept. Biological Chemistry, Harvard Medical School and Dept. Biochemistry and Nutrition, Tufts College Medical School, Boston.*
6. Acetylation in diabetic rats. *Frixos Charalampous* and D. Mark Hegsted. Dept. of Nutrition, Harvard School of Public Health and Dept. Biological Chemistry, Harvard Medical School, Boston.*
7. Metabolism of tryptophan labeled with radioactive carbon. *Dattareya Rao Sanadi* and David M. Greenberg, Division of Biochemistry, Univ. of California Medical School, Berkeley.*
8. Interrelation of age, serum cholesterol and basal metabolism of women. *Mary R. Gram* and Ruth M. Leverton. Human Nutrition Research Laboratory, Univ. of Nebraska, Lincoln.*
9. The effect of various degrees of starvation on gonadotrophic and lactogenic content of rat pituitaries. *Joseph Meites and J. O. Reed (introduced by C. F. Huffman). Depts. Physiology and Pharmacology, Michigan State College, East Lansing.*
10. The nutrition of women during normal and abnormal pregnancy in Panama and the Canal Zone. *Martha J. Thomason*, Nevin S. Scrimshaw* Robert P. Bays* and Estelle E. Hawley. Dept. Obstetrics and Gynecology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*
11. Biochemical studies on primigravidae on self-selected diets which differed greatly in nutritive value. *Helen Oldham, Bernice Blum Sheft* and Thelma Porter. Dept. Home Economics, Univ. of Chicago.*
12. Nutritional evaluation of the iso-oleic acids. I. Elaidic, petroselinic and petroselinelaidic acids. *Henry Sherman*, Laura M. Campling*, Willy Lange* and Robert S. Harris. Nutritional Biochemistry Laboratoris, Massachusetts Institute of Technology, Cambridge.*

THURSDAY, APRIL 21, 4:30 P.M.

Business Meeting

LODGE ROOM B

THURSDAY, APRIL 21, 8:00 P.M.

Symposium on Nutrition in Preventive Medicine

HOTEL STATLER BALLROOM

C. G. King, *Chairman*

1. Nutrition and dental caries. *James H. Shaw. Harvard Univ., Boston.*
2. Human amino acid requirements. *William C. Rose, Univ. of Illinois, Urbana.*
3. Chemical measurements in relation to physical evidence of malnutrition. *Grace A. Goldsmith. Tulane Univ., New Orleans.*
4. Nutrition, renal lesions and hypertension. *Charles H. Best and W. S. Hartroft. Univ. of Toronto, Canada.*
5. Anemias caused primarily by malnutrition. *W. Henry Sebrell. National Institute of Health, Bethesda, Md.*

FRIDAY, APRIL 22, 9:00 A.M.

Minerals; General

LODGE ROOM B

1. Use of radioactive phosphorus in the study of caries resistant and susceptible albino rats. *Ray L. Shirley* and Carl A. Hoppert. Kedzie Chemical Laboratory, Michigan State College, East Lansing.*
2. Perspiration as a factor influencing the iron requirement. *Frances A. Johnston and Gladys C. Hagan*. New York State College of Home Economics, Cornell Univ., Ithaca.*
3. Iron requirements of six adolescent girls. *Doretta Schlaphoff* and Frances A. Johnston. New York State College of Home Economics, Cornell Univ., Ithaca.*
4. Age and calcium utilization. *C. M. McCay, F. E. Lovelace*, C. H. Liu* and G. Kane*. Laboratory of Animal Nutrition, Cornell Univ., Ithaca.*
5. Effect of a gossypol on the body weight of rats. *Alfred H. Free and Helen M. Free*. Miles Research Laboratories, Elkhart, Ind.*
6. Comparison of sugars in the purified diet of baby pigs. *S. R. Johnson, Dept. Animal Industry, Univ. of Arkansas, Fayetteville.*
7. Influence of heredity on the crude protein and carotene contents of corn. *L. W. Aurand* and R. C. Miller. Dept. Agricultural and Biological Chemistry, Pennsylvania State College, State College.*
8. Studies on the function of the ceca in the domestic fowl. *J. R. Couch*, D. R. Knight*, Harry German* and P. B. Pearson. Agricultural and Mechanical College of Texas, College Station.*
9. Distribution and some properties of the mouse salmonellosis resistance factor. *Howard A. Schneider. Laboratories of the Rockefeller Institute for Medical Research, New York City.*
10. Unidentified vitamins of the B complex required by certain insects. *G. Fraenkel (introduced by H. E. Carter). Dept. Entomology, Univ. of Illinois, Urbana.*
11. Protein and amino acid requirements of the German cockroach. *J. L. Noland* and C. A. Baumann. Dept. Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison (Biochem.).*
12. Observations on an unidentified dietary factor concerned in human growth. *Selma E. Snyderman*, Rosa Lee Nemir* and L. Emmett Holt, Jr. Dept. Pediatrics, New York Univ. and the Children's Medical Service, Bellevue Hospital, New York City.*

FRIDAY, APRIL 22, 1:30 P.M.

General

LODGE ROOM B

1. Determination of SLR factor in the liver and urine of rats fed on various diets. *Bacon F. Chow, Lois Hall*, Shirley de Biase* and Lois Barrows*. Division of Pharmacology, Squibb Institute for Medical Research, New Brunswick, N. J.*
2. Use of enriched cereals in certain diets consumed in the South. *Harriet A. Harlin, Beulah D. Westerman (introduced by Martha M. Kramer). Dept. Foods and Nutrition, Kansas Agricultural Experiment Station, Kansas State College, Manhattan.,*
3. Nutrition of *L. bifidus*, predominant intestinal organism of the breast fed infant. *R. M. Tomarelli*, R. F. Norris*, Paul Gyorgy. Univ. of Pennsylvania Hospital, Philadelphia, J. B. Hassinen*, and F. W. Bernhart*, Wyeth, Inc., Mason, Mich.*
4. Influence of dietary protein and fat on the toxicities of trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT) for the rat. *Maurice E. Shils and Leonard J. Goldwater (introduced by C. G. King). Division of Industrial Hygiene, School of Public Health, Columbia Univ., New York City.*

5. Germ-free chick nutrition. I. Development on synthetic-type diets. *J. A. Reyniers, T. D. Luckey, P. C. Trexler, R. F. Ervin, M. Wagner and H. A. Gordon. Germ-Free Life Division, Lobund, Notre Dame, Indiana, R. A. Brown, G. J. Mannering and C. J. Campbell, Research Laboratories, Parke, Davis and Company, Detroit.*
6. Influence of high levels of fat with suboptimal levels of riboflavin on the growth of chicks. *Raymond Reiser* and P. B. Pearson. Agricultural and Mechanical College of Texas, College Station (Biochem.).*
7. Tryptophane requirement of the rat as related to dietary level of other amino acids. *W. D. Salmon. Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn (Biochem.).*
8. Food packages as a supplement to the German nutrition. *G. Lehmann (introduced by D. B. Dill). Max Planck-Institut für Arbeitsphysiologie, Dortmund, Germany (Physiol.).*
9. Ammonium persulfate as a flour maturing agent. *Aaron Arnold and Frans C. Goble*. Sterling-Winthrop Research Institute, Rensselaer.*
10. Studies on prolamines treated with nitrogen trichloride. I. Fractionation of hydrolysates. *L. Reiner, Philip Weiss*, Fernanda Misani*, M. G. Cordasco* and T. W. Fair*. Research Laboratories of Wallace and Tiernan Products, Inc. (Biochem.).*
11. Studies on prolamines treated with nitrogen trichloride. III. Sulfated zein. *Fernanda Misani*, Philip Weiss*, M. G. Cordasco*. T. W. Fair* and L. Reiner. Research Laboratories of Wallace and Tiernan Products, Inc. (Biochem.).*

Papers Read by Title

1. Effect of diet on the treatment of parathyroidectomized dogs. *Aaron Arnold. Sterling-Winthrop Research Institute, Rennsselaer.*
2. Influence of vitamin E on the creatine and creatinine excretion of rats poisoned with CCl_4. *E. L. Hove. Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn.*
3. Color measurement in products of rice milling. *Marinus C. Kik. Univ. of Arkansas, Fayetteville.*
4. Goiter in West Virginia. *Isidor Greenwald. Dept. Chemistry, New York Univ. College of Medicine, New York City.*

THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Thirty-third Annual Meeting

An asterisk following an author's name denotes "by invitation"

TUESDAY, APRIL 19, 9:00 A.M.

LODGE ROOM D

1. 'Nonspecific' complement-fixing activity of cloudy avian sera. *Christine E. Rice. Dominion Dept. of Agr., Animal Diseases Research Institute, Hull, Quebec, Canada.*
2. Antigenicity of staphylocoagulase. *Morris Tager and Harriet B. Hales*. Dept. Bacteriology, Yale Univ. School of Medicine, New Haven.*
3. Some properties of purified diphtheria toxoid. *Leo Levine and Louise Wyman (introduced by Geoffrey Edsall). Antitoxin and Vaccine Laboratory, Mass. Dept. of Public Health, Boston.*
4. Studies on the enzymatic hydrolysis of the capsular polysaccharide of *Klebsiella pneumoniae* Type B. *Edgar E. Baker* and Walther F. Goebel. The Rockefeller Institute for Medical Research, New York City.*
5. Effect of lithium periodate on crystalline bovine serum albumin. *Gertrude E. Perlmann* and Walther F. Goebel. The Rockefeller Institute for Medical Research, New York City.*
6. Polysaccharides isolated from tissues of normal, immune, and immunologically paralyzed mice. *Lloyd D. Felton, Benjamin Prescott*, Gladys Kauffmann*, and Barbara Ottinger*. National Institutes of Health, Bethesda, Md.*
7. Dissociation of antigen-antibody complexes and their bond energy. *Felix Haurowitz and Lale Etili*. Dept. Chemistry, Indiana Univ., Bloomington, and Dept. of Medical Chemistry, Istanbul, Turkey.*
8. Equilibrium studies on the reaction of antibody with hapten. *Leonard Lerman (introduced by Dan H. Campbell). Gates and Crellin Lab. of Chemistry, Calif. Inst. of Technology, Pasadena.*
9. Purification of antibodies by specific methods. *Dan H. Campbell and Leonard Lerman*. Gates and Crellin Lab. of Chemistry, Calif. Inst. of Technology, Pasadena.*
10. Immunochemical studies of factors modifying interaction of egg albumin and rabbit anti-egg albumin. *Walter J. Kleinschmidt* and Paul D. Boyer, Division of Biochemistry, Univ. of Minnesota, St. Paul (Biochem.).*
11. Presidential Address: *Michael Heidelberger.*

TUESDAY, APRIL 19, 2:00 P.M.

LODGE ROOM D

1. Precipitin tests for the diagnosis of Chagas' disease, with special reference to the specificity of polysaccharides. *A. Packchanian and L. Turck*. Dept. Bacteriology and Lab. of Microbiology, Univ. of Texas School of Medicine, Galveston.*
2. Further study of toxins and antigens of *Shigella dysenteriae*. *Daniel A. Boroff and Beatrice P. Macri*. Dept. Bacteriology, Camp Detrick, Frederick, Md., and George Washington Univ., Washington, D. C.*
3. Comparative response and persistence of immunity in mice vaccinated intraabdominally or by gavage with *Shigella sonnei*. *Merlin L. Cooper, Helen M. Keller and Agnes M. Hart (introduced by Albert B. Sabin). Children's Hospital Research Foundation and Dept. Pediatrics, College of Medicine, Univ. of Cincinnati.*
4. Prophylactic immunity to experimental enteric cholera. *William Burrows and Lawrence Ware*. Dept. Bacteriology and Parasitology, Univ. of Chicago, Chicago.*
5. Resistance to endotoxins of enteric bacilli in patients convalescent from typhoid and paratyphoid fever. *Herbert R. Morgan* and Franklin A. Neva (introduced by Thomas Francis, Jr.). Dept. Epidemiology, School of Public Health, Univ. of Michigan, Ann Arbor.*
6. Immunochemical studies on egg white proteins. *L. R. Wetter, M. Cohn and H. F. Deutsch (introduced by A. M. Pappenheimer, Jr.). Dept. Physiological Chemistry, Univ. of Wisconsin Medical School, Madison, and Dept. Microbiology, New York Univ. College of Medicine, New York City.*
7. Demonstration, occurrence and significance of iso-immunization against the A and B factors during pregnancy. *Ernest Witebsky. Dept. Bacteriology and Immunology, Medical School, Buffalo, N. Y.*
8. Effects of peroxidase on the RH-antibodies. *Philip F. Wagley*, Irwin W. Sizer, Louis K. Diamond* and Fred R. Allen*. Dept. Biology, Massachusetts Inst. of Technology, Cambridge, and Blood Grouping Laboratory, Children's Medical Center, Boston (Physiol.).*
9. New observations on an autoagglutinin. *H. H. Lubinski and D. Mendel*. Pathological Labo-*

ratories and Dept. Medicine, Jewish General Hospital, Montreal, Canada.

10. Antisheep agglutinins in infectious mononucleosis. *I. Davidsohn and Chiyo Kashiwagi*. Mt. Sinai Medical Research Foundation and Chicago Medical School.*

TUESDAY, APRIL 19, 8:00 P.M.

Joint Session of the Federation

AUDITORIUM

Program on page 418

WEDNESDAY, APRIL 20, 9:00 A.M.

LODGE ROOM D

1. Inactivation of influenza virus by oxidizing agents. *Robert R. Wagner and Irven B. Stacy, Jr. (introduced by LaVerne A. Barnes). Naval Medical Research Institute, Bethesda, Md.*
2. Amino acids, analogues and the propagation of Theiler's GD VII virus in mouse brain tissue culture. *Harold E. Pearson and Richard J. Winzler*. Dept. Bacteriology and Biochemistry, Univ. of Southern California School of Medicine, and the Laboratory Division, Los Angeles County Hospital, Los Angeles.*
3. Immunologic relations and comparative invasiveness of strains of poliomyelitis from recent epidemics. *Gordon C. Brown*, Thomas Francis, Jr. and John D. Ainslie*. Dept. Epidemiology, School of Public Health, Univ. of Michigan, Ann Arbor.*
4. Effect of previous experience with homologous and heterologus types of poliomyelitis virus upon the alimentary carriage of virus by the chimpanzee. *Howard A. Howe*, David Bodian* and Isabel M. Morgan. Poliomyelitis Research Center, Dept. Epidemiology, Johns Hopkins Univ., Baltimore.*
5. An immunogenic difference between two related strains of poliomyelitis virus. *Joseph L. Melnick. Section of Preventive Medicine, Yale Univ. School of Medicine, New Haven.*
6. Antibody against encephalomyocarditis virus in the sera of American wild rats. *Joel Warren, Sudie B. Russ, Helen Jeffries (introduced by Joseph E. Smadel). Army Medical Dept., Res. and Grad. School, Army Medical Center, Washington, D. C.*
7. Antigenic relationship of dengue and yellow fever viruses with those of West-Nile and Japanese B encephalitis. *Albert B. Sabin. Children's Hospital Research Foundation, Univ. of Cincinnati College of Medicine.*
8. Difference in antigenic capacity among influenza virus strains. *Jonas E. Salk, Angela M. Laurent* and Rachel C. McGinnis*. Virus Research Laboratory, Dept. Bacteriology, Univ. of Pittsburgh School of Medicine.*
9. An indirect complement-fixation test for psittacosis-lymphogranuloma venereum group antibodies in chicken antisera. *M. R. Hilleman and R. J. Helmold (introduced by Annabel W. Walter). Army Medical Dept., Res. and Grad. School, Army Medical Center, Washington, D. C.*
10. Neutralization technique in tsutsugamushi disease (scrub typhus) and the antigenic differentiation of rickettsial strains. *John P. Fox (introduced by P. K. Olitsky). International Health Board, Rockefeller Foundation, New York City.*

WEDNESDAY, APRIL 20, 2:00 P.M.

LODGE ROOM D

1. Development of the specific antibody for a distinctive constituent of Brown-Pearce carcinoma cells. *Bernard Kalfayan* and John G. Kidd. Dept. Pathology, New York Hospital-Cornell Meeical Center, New York City.*
2. Metabolism of Brown-Pearce rabbit carcinoma cells in normal and immune serum. *W. H. Summerson* and John G. Kidd. Dept. Biochemistry and Pathology, Cornell Univ. Medical College, New York City.*
3. Effects of gliotoxin and other sulfur-containing compounds on tumor cells in vitro. *John W. Mason* and John G. Kidd. Dept. Pathology, New York Hospital-Cornell Medical Center, New York City.*
4. Tuberculostatic activity of the blood and urine of animals given gliotoxin. *Ralph Tompsett*, Walsh McDermott* and John G. Kidd. Dept. Medicine and Pathology, New York Hospital-Cornell Medical Center, New York City.*
5. Evidence for the controlling influence of protease-inhibitors in allergic phenomena. *Kenneth L. Burdon, Dept. Bacteriology and Immunology, Baylor Univ. College of Medicine, Houston.*
6. Loss of hemolytic complement and of granulo-

cytes following reinjection of an antigen into the rabbit. *A. B. Stavitsky*, R. Stavitsky* and E. E. Ecker. Institute of Pathology, Western Reserve Univ. Cleveland.*

7. Anaphylactic studies in mice. *A. H. Wheeler, E. Brandon and H. Petrenco (introduced by Reuben L. Kahn). Serology Laboratory, Univ. Hospital, Ann Arbor.*
8. Interference with induction of the anaphylactic state by prior feeding of a hapten-like allergenic chemical. *Merrill W. Chase. Rockefeller Institute for Medical Research, New York City.*
9. An allergenic fraction of ragweed pollen prepared by electrophoresis-convection (Nielsen-Kirkwood Cell). *Mary Hewitt Loveless and Robert Best*. New York Hospital, and Dept. Medicine, Cornell Univ. Medical College, New York City.*
10. Role of cathepsin II in the mechanism of caseation in experimental tuberculosis of rabbits. *Charles Weiss and Julius Schultz*. Laboratories of Jewish Hospital, Philadelphia.*

WEDNESDAY, APRIL 20, 7:45 P.M.

Symposium on Mechanisms of Immunity to Viruses

LODGE ROOM D

1. Joseph W. Beard, *Chairman*. Duke Univ., Durham.
2. Joseph M. Morgan. Johns Hopkins Univ., Baltimore.
3. R. Walter Schlesinger. Public Health Research Inst., New York City.
4. Jules Freund. Public Health Research Inst., New York City.
5. Albert B. Sabin. Univ. of Cincinnati.
6. John F. Enders. Harvard Medical School, Boston.
7. Douglas H. Sprunt. Univ. of Tenn., Memphis.
8. S. G. Anderson. Western Reserve Univ., Cleveland.
9. Frank Lanni. Duke Univ. School of Medicine, Durham.
10. Werner Henle. Univ. of Pa., Philadelphia.

THURSDAY, APRIL 21, 9:00 A.M.

LODGE ROOM D

1. Relation of complement to thermolabile hyaluronidase inhibitor of serum. *Stanley Marcus and John K. Fulton.* Rackham Arthritis Research Unit and Dept. Medicine, Univ. of Michigan, Ann Arbor.*
2. A comparison of methods of determining differences in virulence for mice of strains of *salmonella typhosa. H. C. Batson, Marucie Landy and Martha Brown (introduced by Marion C. Morris). Army Medical Dept., Res. and Grad. School, Army Medical Center, Washington, D. C.*
3. Relationship between catalase activity and virulence in *pasteurella pestis. Morris Rockenmacher (introduced by A. P. Kruger). Office of Naval Research Task V, Dept. Bacteriology and U. S. Naval Medical Research Unit No. 1, Univ. of California, Berkeley.*
4. Histochemical evidence for the synthesis of protein by lymphocytes following parenteral injection of antigen. *T. N. Harris and Susanna Harris*. Children's Hospital of Philadelphia, Dept. Pediatrics, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
5. Effect of x-irradiation on antibody decline. *William H. Taliaferro and Lucy G. Taliaferro*. Dept. Bacteriology and Parasitology, Univ. of Chicago.*
6. Streptococcal anti-hyaluronidase in rheumatic fever. *Susanna Harris* and T. N. Harris. Children's Hospital of Philadelphia, Dept. Pediatrics, School of Medicine, Univ. of Pennsylvania, Philadelphia.*
7. Universal serologic reaction with lipid antigen in tuberculosis. *Reuben L. Kahn. Serology Laboratory, Univ. Hospital, Ann Arbor.*
8. Intradermal antibody-antigen and antigen-antibody reactions in persons having poliomyelitis, contacts and non-contacts in relation to poliomyelitis. *Edward C. Rodenow. Bacteriological Research, Longview Hospital, Cincinnati.*
9. Procaine penicillin and sulfonamide antagonism. *H. Fishback*, H. Welch, E. Q. King*, J. Levine*, C. W. Price and W. A. Randall. Federal Security Agency, Food and Drug Administration, Washington, D. C.*

THURSDAY, APRIL 21, 11:45 A.M.

Immunology Business Meeting

LODGE ROOM D

THURSDAY, APRIL 21, 2:00 P.M.

Symposium on Serology of Blood Groups

LODGE ROOM D

Chairman; Louis Diamond. Harvard Medical School, Boston

Papers Read by Title

1. Tissue reaction to foreign particulate matter altered by presence of absorbed antigen. *Howard C. Hopps and Alfred M. Shideler*. Dept. Pathology, Univ. of Oklahoma School of Medicine, Oklahoma City.*
2. Relationship of blood sugar and hypoproteinemia to antibody response in diabetics. *Michael G. Wohl, S. O. Waife, Stanley Green, and George B. Clough (introduced by Max B. Lurie). Philadelphia General Hospital.*
3. Quantitative measurement of antibody to a whole bacterial antigen. *Marian Koshland (introduced by William Burrows). Dept. Bacteriology and Parasitology, Univ. of Chicago.*
4. Fibrinolysin activation by specific antigen, peptone and polysaccharides. *Goerges Ungar (introduced by A. A. Schiller). Rheumatic Fever Research Institute, Northwestern Univ., Chicago (Physiol.).*
5. Passive sensitization of normal dogs by thoracic duct lymph from dogs sensitized to horse serum *Parke H. Woodard (introduced by K. E. Jochim). Dept. Physiology, Univ. of Kansas, Kansas City (Physiol.).*

EXHIBITORS

The Technical Exhibits will be located in the Masonic Temple, Chrystal Ballroom. They will be open Monday 2:00 to 5:00 p.m.; Tuesday, 9:00 a.m. to 5:00 p.m. and 7:00 to 10:00 p.m.; Wednesday and Thursday, 9:00 a.m. to 5:00 p.m. The following firms supplying the needs of biologists are represented and have prepared exhibits of much interest and value. Your time in visiting these will be well repaid.

A. S. Aloe Company, St. Louis, Mo. (*Booths 36, 37, 38*)
American Instrument Company, Inc., Silver Spring, Md. (*Booth 41*)
American Optical Company, Scientific Instrument Division, Buffalo, N. Y. (*Booths 33, 34*)
Ames Company, Inc., Elkhart, Ind. (*Booth 12*)
Appleton-Century-Crofts, Inc., New York City. (*Booth 6*)
Armour and Company, Chicago, Ill. (*Booth 40*)
Army Chemical Center, Medical Division, Md. (*Booths 18, 19*)
Bilhuber-Knoll Corp., Orange, N. J. (*Booth 32*)
Biological Abstracts, University of Pennsylvania, Philadelphia, Pa. (*Booth 26*)
Ernst Bischoff Company, Inc., Ivoryton, Conn. (*Booth 59*)
The Blakiston Company, Philadelphia, Pa. (*Booth 23*)
Camel Cigarettes, New York City. (*Booths 43, 44*)
Central Scientific Co., Chicago, Ill. (*Booth 4*)
Cinchona Products Institute, New York City. (*Booth 51*)
Clay-Adams Company, Inc., New York City. (*Booth 13*)
The Coca-Cola Company, Atlanta, Ga. (*Coca-Cola Lounge*)
Coreco Research Corporation, New York City. (*Booth 35*)
Gamma Instrument Company, Inc., New York City. (*Booth 7*)
Grass Instrument Company, Quincy, Mass. (*Booth 27*)
Grune & Stratton, Inc., New York City. (*Booth 14*)
Heiland Research Corporation, Denver, Colo. (*Booth 21*)
H. T. Hernberg, Philadelphia, Pa. (*Booth 29*)
Paul B. Hoeber, Inc., New York City. (*Booth 1*)
Lea & Febiger, Philadelphia, Pa. (*Booth 39*)
Lederle Laboratories Division, American Cyanamid Company, New York City. (*Booth 3*)
J. B. Lippincott Company, Philadelphia, Pa. (*Booths 53, 54*)
The Macmillan Company, New York City. (*Booth 15*)
McGraw-Hill Book Company, Inc., New York City. (*Booth 5*)
Merck & Co., Inc., Rahway, N. J. (*Booths 8, 9*)
The C. V. Mosby Company, St. Louis, Mo. (*Booths 55, 56*)
Phipps & Bird, Inc., Richmond, Va. (*Booth 17*)
Rahm Instruments, Inc., New York City. (*Booth 25*)
The Rayoscope, Delaware, Ohio. (*Booth 10*)
Sanborn Company, Cambridge, Mass. (*Booth 50*)
Sandoz Pharmaceuticals, New York City. (*Booths 47, 48*)
W. B. Saunders Company, Philadelphia, Pa. (*Booth 16*)
E. R. Squibb & Sons, New York City. (*Booth 52*)
The Technicon Company, New York City. (*Booths 57, 58*)
Charles C Thomas—Publisher, Springfield, Ill. (*Booth 2*)
Tracerlab, Inc., Boston, Mass. (*Booth 45*)
University Presses, Chicago, Ill. (*Booth 49*)
Varick Pharmacal Company, Inc., New York City. (*Booth 42*)
The Year Book Publishers, Inc., Chicago, Ill. (*Booth 11*)

DIRECTORY OF COUNCIL AND COMMITTEE MEETINGS AND SPECIAL FUNCTIONS

Sunday, April 17

Federation Executive Committee: (6:00 P.M. (*Suite 808, Book-Cadillac*)

Physiology, Council: 9:00 A.M.; 1:00 P.M.; 7:00 p.m. (*Room B, Statler*)

Pharmacology, Council: 10:00 A.M.; 1:00 P.M.; 7:00 P.M. (*Room C, Statler*)

Nutrition, Council: 1:00 P.M. (*Room D, Statler*)

Monday, April 18

Federation Proceedings Control Committee: 9:00 A.M. (*Parlor J, Book-Cadillac*)

Federation Executive Committee: 3:00 P.M. (*Suite 808, Book-Cadillac*)

Physiology, Council: 9:00 A.M.; 1:00 P.M. (*Room A, Statler*)

Pharmacology, Council: 10:00 A.M. (*Room B, Statler*)

Pathology, Council: 10:00 A.M. (*Suite 808, Book-Cadillac*)

Nutrition, Council: 9:00 A.M.; 1:00 P.M.; 7:00 P.M. (*Room E, Statler*)

Immunology, Council: 10:00 A.M.; 8:00 P.M. (*Parlor K, Book-Cadillac*)

American Association of Biological Chemists, Committee on Clinical Chemistry: 10:00 A.M.; 1:30 P.M. (*Suite 500, Book-Cadillac*)

Pharmacology Society, Editorial Board: 10:00 A.M. (*Room F, Statler*)

Annual Review of Physiology, Editorial Meeting: 2:00 P.M. (*Room A, Statler*)

Pharmacological Reviews, Editorial Conference: 10:00 A.M. (*Salle Detroit, Statler*)

Society for Experimental Biology and Medicine, Membership Committee: 4:30 P.M. (*Room D, Statler*)

Food and Nutrition Board, National Research Council: 9:30 A.M. (*Parlor F, Book-Cadillac*)

Poultry Nutrition Discussion Group: 2:00 P.M.; 7:00 P.M. (*Italian Garden, Book-Cadillac*)

LUNCHEONS

Society for Experimental Biology and Medicine, Editorial Board: 12:30 P.M. (*Room D, Statler*)

DINNERS

Society for Experimental Biology and Medicine, Council: 6:15 P.M. (*Room F, Statler*)

Journal of Nutrition, Editorial Board: 6:30 P.M. (*Room D, Statler*)

Nutrition Reviews, Editorial Board: 5:00 P.M. (*Parlor F, Book-Cadillac*)

Tuesday, April 19

Physiology Society, Editorial Conference: 6:30 P.M. (*Masonic Temple Auditorium*)

Society for Experimental Biology and Medicine, Advertising Committee: 9:30 A.M. (*Room A, Statler*); Secretaries Meeting, 10:30 A.M. (*Room A, Statler*); Finance Committee, 1:00 P.M. (*Room E, Statler*)

LUNCHEONS

University of Illinois Alumni: 12:15 P.M. (*English Room, Book-Cadillac*)

DINNERS

Journal of Biological Chemistry, Editorial Board: 6:00 P.M. (*Founders' Room, Book-Cadillac*)

Wednesday, April 20

Physiology, Council: (time to be arranged) (*Room D, Statler*)

Pharmacology, Council: 10:00 A.M. (*Room E, Statler*)

Biological Synthesis and Radio-active Materials, Informal Discussion Group: 2:00 P.M. (*Ivory Room, Statler*)

Nutrition of the Mouse: Symposium: 7:30 P.M. (*Ivory Room, Statler*)

BREAKFAST

University of Chicago Home Economics Alumnae: 7:30 A.M. (*Room B, Statler*)

LUNCHEONS

Former Army Nutrition Officers: 12:00 noon *Pan American Room, Book-Cadillac*)

DINNERS

Biochemical Preparations Group: 6:30 P.M. (*Suite 500, Book-Cadillac*)

Pathology Society, President's Dinner: 6:30 P.M. (*Founders' Room, Book-Cadillac*)

*Temperature Regulation Section, Physiology Society: 5:30 P.M. (*Normandie Room, Book-Cadillac*)

*Attendance by invitation.

Thursday, April 21

Federation Executive Committee: 9:00 P.M. (*Suite 808, Book-Cadillac*)

Physiology, Council: 7:00 P.M. (*Room B, Statler*)

Pathology, Council: 7:00 P.M. (*Suite 808, Book-Cadillac*)

Nutrition, Council: 7:00 P.M. (*Room E, Statler*)

Immunology, Council: 7:00 P.M. (*Parlor K, Book-Cadillac*)

TEA

Afternoon Tea, 4:00–6:00 P.M., at the Student Center, Wayne University, for all women members and guests

DINNERS

*Bureau of Biological Research, Rutgers University: 6:30 P.M. (*Room C, Statler*)

*Circulation Section, Physiology Society: 6:30 P.M. (*English Room, Book-Cadillac*)

Immunology Society, Editorial Board: 6:30 P.M. (*Room D, Statler*)

*Metabolism Discussion Group: 6:30 P.M. (*Normandie Room, Book-Cadillac*)

---

* Attendance by invitation.

INDEX OF AUTHORS

A

Aaness, R. J. 486
Abbott, L. D., Jr. 455
Abdou, I. A. 476
Abell, L. L. 448
Abood, L. G. 467, 470
Abramson, S. 480
Abreu, B. E. 476
Adams, W. E. 425
Addis, T. 443
Ades, H. W. 427
Adler, H. F. 427
Adler, T. K. 476
Aegerter, E. A. 421
Agner, K. 456
Ahlquist, R. P. 465, 476
Ahmad, K. 454
Ainslie, J. D. 492
Albanese, A. A. 455
Albaum, H. 451
Albaum, H. G. 459
Albert, R. E. 438
Alburn, H. E. 457
Alexander, B. 439
Alexander, J. D. 440
Alexander, R. S. 423
Algire, G. H. 437
Allen, J. G. 436, 439
Allen, F. R. 491
Allen, F. W. 460
Allen, T. H. 429
Allen, W F. 430
Alles, G. A. 440
Alling, E. L. 483
Allison, J. B. 482
Alper, C. 460
Alsdorf, W. R. 474
Altland, P. D. 426
Altman, K. I. 433, 459, 481
Alvarez, E. 473
Alving, A. S. 445
Amador, L. V. 430
Ambrose, A. M. 472
Amdur, M. O. 460
Ames, A. 428
Ames, S. 475
Amoroso, M. D. 443
Ananenko, E. 463
Anderson, C. E. 460
Anderson, E. 438
Anderson, G. C. 487
Anderson, H. H. 474
Anderson, J. T. 485
Anderson, R. C. 476
Anderson, S. G. 493
Andronis, A. 437
Angelone, L. 443
Angerer, C. A. 421, 443
Anguiano, G. 440
Anigstein, L. 464
Annegers, J. H. 433
Ansbacher, S. 486
Anslow, W. P. 425
Anthony, D. S. 454
Antia, F. 438
Aposhian, H. V. 458
Archer, S. 468
Archibald, R. M. 453
Arends, R. L. 470
Armstrong, W. D. 453
Arnold, A. 490
Arscott, P. M. 421
Arsove, S. 448
Artom, C. 454, 460
Asenjo, C. F. 447
Ashwell, G. 443
Atchley, W. 451
Atkinson, W. B. 429
Auerbach, M. E. 474
Auerbach, V. H. 455
Aurand, L. W. 489
Avera, J. W. 431, 440
Aviado, D. M. 419, 434
Axelrod, J. 463, 471

B

Bacchus, H. 420, 443
Bach, L. M. N. 427
Bacher, J. E. 460
Bader, M. E. 438
Bader, R. A. 425
Baez, S. 425
Baker, E. E. 491
Baker, H. M. 423
Bakst, H. 467
Balaban, M. 422
Baldes, E. J. 418
Baldwin, D. S. 445
Bale, W. F. 459
Bales, P. D. 418, 421
Ball, S. M. 428
Balls, A. K. 458
Banfield, W. G., Jr. 470
Bang, F. B. 479
Barber, M. A. 456
Bardwell, K. 419
Barker, E. S. 419
Barker, H. G. 431
Barker, S. B. 429
Barnes, R. H. 487
Barnes, T. C. 421, 443, 464
Barnes, T. R. 477
Barnett, H. L. 425
Barnett, S. R. 459
Barnum, C. P. 459
Barrett, W. 470, 472
Barron, D. H. 419
Barrows, L. 489
Bartlett, P. D. 447
Bass, A. D. 456
Bass, D. E. 425
Basinski, D. H. 456
Bassett, R. C. 431
Batson, H. C. 493
Batterman, R. C. 471
Battista, A. F. 427
Bauer, R. O. 468
Baumann, E. J. 454
Baumann, C. A. 485, 489
Baumberger, J. P. 419
Bay, E. B. 425
Bays, R. P. 488
Bazett, H. C. 420
Beach, E. F. 460
Bean, J. W. 422, 442
Beard, J. W. 493
Beckman, H. 476
Becklund, W. W. 474
Begany, A. J. 465
Beher, W. T. 453
Behrmann, V. G. 425
Beinert, H. 480
Belford, J. 473
Bell, R., Jr. 427
Bell, R. G. 473
Belogorsky, J. B. 465
Belt, M. 447
Bender, M. B. 427
Bendich, A. 456
Benditt, E. P. 482, 483
Benedict, J. D. 456
Beninson, J. 464
Benjamin, Z. H. 467
Bennett, L. L. 443
Bennett, L. R. 481
Bennett, V. C. 481
Bennett, W. D. 468
Berger, A. R. 433
Berger, E. Y. 434
Berger, F. M. 470
Berghout, J. 477
Bergner, A. D. 421
Berke, N. 476
Berliner, R. W. 428, 453
Bernhart, F. W. 489
Bernheim, F. 460, 476
Bernhein, M. L. C. 460
Bernstein, D. 423
Bernstein, E. 463
Berry, C. 477
Bessman, S. P. 447
Best, C. H. 442, 489
Best, R. 493
Beutner, K. R. 476
Beutner, R. 421, 464, 478
Bibb, J. C. 477
Bickford, R. G. 430
Bieri, J. G. 449
Bieter, R. N. 474
Bilskei, R. 438
Bing, R. J. 426
Bingham, W. G. 432
Binkley, F. 455
Bird, H. R. 485
Bird, J. G. 472, 473
Bird, R. M. 421
Birnie, J. H. 428, 443
Bishop, C. W. 433
Bitman, J. 453
Bjorkman, G. N. 421
Black, H. 419
Blake, W. D. 431
Blaylock, L. G. 487
Blickenstaff, D. 439
Bliss, C. I. 469
Bloch, K. 449
Block, R. J. 448
Bluemle, L. W., Jr. 460
Blumer, A. 467
Bly, C. G. 488
Bobb, A. L. 467
Bodansky, O. 455, 456
Bodian, D. 492
Bodine, J. H. 421
Bohr, D. F. 437, 440
Bohr, F. 450
Boldrey, E. B. 466
Bolene, C. 440
Bolling, D. 448
Bollman, J. L. 428, 455, 482, 483
Bolomey, A. A. 425
Bondy, P. K. 449
Bonnycastle, D. D. 463, 473
Bonsal, C. 456
Booker, W. M. 423, 463, 487
Booth, C. 465
Bordeaux, J. 441
Borden, A. 485
Borek, E. 447
Borge, A. F. 432
Borison, H. L. 435
Boroff, D. A. 491
Borsook, H. 452
Boss, W. R. 428, 443
Bosshardt, D. K. 487
Bott, P. A. 433
Bourque, J. E. 440
Bowen, W. J. 427, 443
Bowles, L. L. 485
Bowman, D. E. 460, 482
Bowman, K. P. 463
Bowman, R. L. 453
Boxer, G. E. 464
Boyarsky, L. L. 435
Boyd, R. I. 443
Boyer, P. D. 449, 491
Boyle, D. 420
Boyle, H. H. 454
Boyles, P. W. 439
Bradbury, J. T. 426
Bradford, S. 442
Bradin, J. L., Jr. 474
Brady, J. 441
Brand, F. C. 448
Brandon, E. 493
Brandt, A. E. 469
Brauer, R. W. 452, 468, 469, 482
Braun, H. A. 467
Brecher, G. A. 419, 427
Breckenridge, C. G. 422
Breed, E. S. 423
Breed, N. D. 451
Breedis, C. 482
Brendler, H. 426
Brewer, N. R. 475
Brewer, W. D. 488
Bridges, W. C. 467
Brill, H. H. 419
Brin, M. 456
Brindley, C. O. 445
Brinegar, M. J. 485
Brinkhous, K. M. 482
Brittain, R. P. 477
Britton, S. W. 443
Brodie, B. B. 434, 448, 463, 471

Brodie, O. J. 478
Brody, D. A. 437
Brookhart, J. M. 427, 432
Broquist, H. P. 455
Brotman, M. 477
Brown, A. E. 469
Brown, B. B. 472
Brown, B. H. 465
Brown, E. 426
Brown, F. A., Jr. 443
Brown, G. B. 456
Brown, G. C. 492
Brown, G. M. 458
Brown, K. D. 458
Brown, M. 493
Brown, R. A. 490
Brown, W. E. 426
Brownlee, K. A. 469
Brozek, J. 437
Brues, A. M. 424, 481
Bruner, H. D. 464
Brunner, M. P. 453
Brust, M. 435
Buchanan, A. R. 418
Bucher, G. R. 438, 460
Bucher, G. R. 461
Buckley, E. S. 432, 439
Buckwalter 482
Budde, J. H. 460
Bueding, E. 452
Buehler, H. J. 457
Buell, M. V. 454, 461
Bumpus, F. M. 454
Burch, B. H. 427
Burchell, H. B. 425
Burckhardt, W. L. 427
Burdon, K. L. 492
Burgoyne, F. H. 479
Burk, D. 454
Burke, J. C. 470
Burns, R. O. 445
Burns, R. O., Jr. 440
Burr, G. O. 457
Burrows, W. 491
Burson, H. 475
Burstein, S. G. 443
Burton, A. C. 431, 439
Butcher, H. R. 434
Butler, A. M. 453
Butler, T. C. 475
Butler, W. W. S. 426
Byerrum, R. U. 468
Byers, S. O. 423

C

Cagan, R. N. 448
Caldwell, J. H. 486
Calesnick, B. 476
Callender, S. 480
Cameron, A. 466, 470, 471
Camp, W. J. R. 465
Campbell, B. 421, 480
Campbell, C. J. 490
Campbell, D. H. 491
Campbell, G. S. 431, 434
Campbell, J. 421
Campling, L. M. 488
Campoy, F. 460
Cannon, P. R. 483
Cantarow, A. 420, 429
Cantrell, W. 475
Canzanelli, A. 421
Capo, L. R. 466
Capps, R. T. 470, 474
Caren, R. 441
Cargill, W. H. 428
Carlson, A. J. 475
Carlson, J. S. 421
Carlson, V. W. 457
Carlson, W. W. 457
Carmichael, E. B. 460
Carr, C. J. 471
Carroll, K. K. 433, 439
Carson, S. F. 454
Carter, C. E. 459, 481
Carter, H. E. 448
Carter, P. 426
Cartwright, G. E. 480
Cary, C. A. 451
Casarett, G. W. 433, 459, 481
Casas, C. B. 426
Cassels, D. E. 434
Cassidy, J. W. 456
Caster, W. O. 453
Castillo, J. C. 470
Catchpole, H. R. 423, 445
Cavanaugh, D. 443
Cavanaugh, D. J. 443
Celmer, W. D. 448
Chadbourn, W. 474
Chalmers, J. H. 431, 437
Chambers, W. W. 430
Chang, H.-T. 427
Chang, M. C. 418
Chang, Y. T. 476
Chanutin, A. 452, 460
Chapman, D. W. 471
Charalampous, F. 488
Chargaff, E. 448, 459
Charipper, H. A. 423
Chase, A. M. 443
Chase, H. B. 424
Chase, J. H. 464
Chase, M. W. 493
Chatfield, P. O. 427
Chen, G. 466, 472
Chen, K. K. 466, 476
Chen, S. D. 487
Cheng, C.-P. 420
Chenoweth, M. B. 468
Chenoweth, M. T. 454
Chernoff, H. M. 429
Child, G. P. 471, 476
Chin, Y. 474
Chinard, F. P. 431
Chinn, H. I. 422
Chittum, J. R. 472
Chow, B. F. 460, 489
Chriss, J. W. 420, 444, 471
Christensen, H. N. 459
Chusid, J. G. 430
Cisler, L. E. 484
Cizek, L. 434
Cizek, L. J. 437
Clapper, W. E. 464
Clark, A. M. 441
Clark, B. B. 466
Clark, G. 430
Clark, J. K. 419, 431
Clark, R. T., Jr. 419
Clark, W. C. 437
Clarke, D. A. 468
Clary, M. L. 444
Clayton, R. M. 488
Clements, J. 480
Clements, R. 465
Clough, G. B. 494
Clowdus, B. F. 460
Cobbey, T. S., Jr. 453
Cochran, K. W. 468
Code, C. F. 437, 439
Coffey, R. E. 487
Cohen, P. P. 452, 458
Cohen, S. 448
Cohen, S. L. 453
Cohn, C. 433, 446
Cohn, D. V. 423
Cohn, M. 491
Cohn, W. E. 459
Cole, V. V. 474
Collier, H. B. 448
Collings, W. D. 431
Colvin, F. M. 423
Conger, A. D. 481
Conger, G. T. 438
Conn, E. C. 451
Connor, A. C. 480
Consolazio, C. F. 488
Constant, G. 437, 443
Cook, M. 441
Cooke, R. E. 453
Cookson, B. A. 418
Cooley, S. L. 486
Coon, M. J. 449
Coon, J. M. 468, 469, 472
Cooper, M. L. 491
Copland, D. E. 442
Copeland, D. H. 486
Copeland, J. E. 470
Copenhaver, N. J. 442
Coppedge, R. L. 484
Corcoran, A. C. 443
Cordasco, M. G. 461, 490
Corey, E. L. 427
Cori, C. F. 457
Cornatzer, W. E. 454
Correll, J. W. 484
Corson, S. A. 425, 471
Coryell, M. N. 460
Cotten, M. deV. 419, 478
Couch, J. R. 489
Coulter, N. A. 431
Cournand, A. 419, 434
Cowgill, G. R. 486
Cowgill, R. C. 448
Cox, R. H. 448
Craig, F. 434
Craig, W. McK. 436
Cranston, E. M. 474
Cranston, R. W. 431, 437
Cravens, W. W. 486
Craver, B. 466, 470, 471, 472
Crismon, J. M. 440
Cronk, L. B. 458
Crookshank, H. R. 460
Cross, R. 451
Crowder, M. 454, 460
Csonka, F. A. 486
Cucci, M. W. 465
Cuchie, F. 464
Cuckler, A. C. 474
Cunningham, M. D. 472
Curtis, G. M. 480
Curtis, H. J. 439

D

Da Costa, E. 488
Dailey, R. E. 478
Damron, C. M. 487
D'Angelo, S. A. 423, 443
Danowski, T. S. 428, 429
Darby, W. J. 488
Darrow, D. C. 453
Davey, B. L. 487
Davidon, B. 468
Davidsohn, I. 492
Davidson, I. W. F. 421
Davies, D. F. 433
Davis, B. D. 451, 455
Davis, H. 427, 476
Davis, J. E. 472, 476
Davis, J. O. 423
Davis, J. P. 476
Davison, C. 468
Day, P. L. 447, 487
Dayrit, C. 467
Dayton, S. 452
de Beer, E. J. 468, 470
de Biase, S. 489
de Bodo, R. C. 420, 443
De Boer, B. 463, 476
De Boer, K. O. 476
DeEds, F. 472
Deering, I. D. 430
Deering, R. C. 420
DeGraff, A. C. 471
DeGusti, D. 474
de Gutierrez-Mahoney, C. G. 430
Deichmann, W. B. 476
Del Campillo, A. 454
Delez, A. L. 455
del Pozo, E. C. 440
Delury, D. B. 469
DeMaria, W. J. A. 423
DeMeio, R. H. 458
Denison, A. B., Jr. 464
Denton, C. A. 485
Desmarais, A. 420
DeSuto-Nagy, G. I. 479
Deutsch, H. F. 450, 460, 491
Dews, P. B. 468
Dexter, L. 420
Deyrup, I. J. 437
Diamond, L. 464, 493
Diamond, L. K. 491
DiBiase, S. 460
Dickman, S. R. 457
Diermeier, H. F. 443
Dietrich, L. S. 447
Dietz, N., Jr. 461
Dill, W. A. 465
Dillard, G. H. L. 448
Dimmitt, J. 442
Dinning, J. S. 487
Di Palma, J. R. 467
Dobriner, K. 453
Dodds, G. A. 444
Doisy, E. A. 457
Dorfman, A. 457
Dornbush, A. C. 447
Dorrance, C. 432
Doty, R. W. 435
Douglas, J. C. 419
Dounce, A. L. 459
Doull, J. 471
Dow, P. 420, 428, 429
Dowling, C. V. 426
Dowling, R. 427
Downing, C. F. 431
Downs, W. L. 465
Doyle, L. P. 455

Drabkin, D. L. 452, 483
Draize, J. H. 473
Draper, W. B. 422, 464, 470
Dreiling, D. A. 442
Dreisbach, R. H. 476
Drekter, L. 488
Drury, D. R. 440
Dryden, L. P. 451
Dryer, R. L. 460
Dso, L 437
Dubach, R. 480
Dubin, I. N. 480
Dubnoff, J. W. 455
DuBois, K. P. 468, 471
Dugal, L. P. 438, 443
Duggan, J. J. 428, 431
Duncanson, D. 430, 431
Dunn, A. L. 476
Dunn, M. C. 464
Dunn, T. B. 490
Dunning, M. F. 434
Dunshee, B. R. 454
Dury, A. 420, 443
duVigneaud, V. 455
Dwoning, J. F. 486
Dyniewicz, H. A. 484
Dziewiatkowski, D. 461

E

Eadie, G. S. 460
Earl, A. 466, 470
Earle, D. P. 440
East, M. K. 455
Easterling, L. 486
Easterwood, M. 453
Ebaugh, F. G., Jr. 436
Eckart, H. N. 474
Eckel, R. 419, 426
Ecker, E. E. 493
Eckstein, R. W. 419, 426
Edelman, I. S. 423, 425
Edelmann, A. 424
Eder, H. A. 431
Ederstrom, H. E. 432
Edholm, O. G. 430, 431, 435
Edison, A. O. 464
Edsall, J. T. 482
Edwards, C. T. 438
Edwards, L. E. 438
Egner, W. 436, 439
Ehrenhaft, J. L. 434
Ehrich, W. E. 465, 483
Eichna, L. W. 433
Eigen, E. 447
Eisenbrandt, L. L. 476
Eisner, H. J. 486
Elam, J. O. 434
Elam, W. N., Jr. 434
Elghammer, R. M. 435, 439
Eliot, J. W. 425
Elkins-Kaufman, E. 458
Elkinton, J. R. 425
Ellinwood, L. E. 469
Elliott, H. W. 466, 476
Ellis, F. W. 470, 476
Elvehjem, C. A. 447, 485, 487
Elwell, L. H. 422
Emele, J. F. 472
Emerson, G. A. 487
Emerson, G. A. 455, 465, 475, 477, 484
Emery, F. E. 426
Enders, J. F. 493
Engel, E. 486
Engel, L. L. 426
Engel, R. W. 487
Ensor, C. R. 466
Epstein, J. A. 435
Erickson, C. C. 483
Ervin, R. F. 490
Eschenbrenner, A. 479
Escher, D. J. W. 423, 425
Essex, H. E. 418
Essex, H. H. 431
Essig, C. F. 418, 421, 444
Estrada, R. L. 482
Etili, L. 491
Ettinger, R. 453
Evans, E. A., Jr. 451
Evans, F. G. 424
Evans, H. 467
Evans, J. D. 421
Everett, G. M. 466
Eversole, W. J. 428, 445
Ewing, P. L. 455, 465, 475, 477, 484

F

Fainer, D. C. 422
Fair, T. W. 461, 490
Fahr, G. 434
Falco, E. A. 449
Farah, A. 428
Farber, S. J. 440
Farber, E. 454
Fardig, O. B. 451
Fassett, D. W. 475, 476
Favre-Gilly, J. 482
Fearl, C. L. 426
Featherstone, R. M. 474
Feickert, J. 472
Feigin, I. 455
Fellows, E. J. 464, 466, 474, 476
Felton, L. D. 491
Fenn, W. O. 419
Fenton, P. F. 486
Feng, P. P. C. 469
Ferguson, C. C. 433, 452
Ferguson, D. 437
Ferguson, F. C., Jr. 473
Ferguson, J. H. 435, 439, 443
Ferguson, J. K. W. 419
Ferry, J. D. 482
Fiala, S. 452
Field, J. 424
Field, L. 467
Finch, C. A. 439
Finch, S. 439
Fincke, M. L. 487
Finkel, M. P. 481
Finlayson, D. M. 419
Finnegan, J. K. 473, 477
Fisch, S. 436
Fischer, E. 442
Fiset, P. E. 443
Fishback, H. 493
Fisher, C. F. S. 439
Fisher, K. C. 424, 432, 440, 442
Fishgold, J. 421
Fishman, A. P. 423
Fishman, W. H. 457
Fiskin, R. A. 464
Firminger, H. I. 482
Fitzgerald, L. R. 421, 424
Fitzhugh, O. G. 467, 468
Fitzpatrick, H. F. 420
Fitzpatrick, T. B. 448
Flanagan, J. B. 423
Flasher, J. 440
Flataker, L. 467, 475
Fleck, S. 432
Fleisher, G. A. 455
Fleisher, J. N. 469
Fleishmann, W. 421
Flickinger, D. 427
Flock, E. V. 455, 483
Flynn, J. E. 482
Folch, J. 448
Follis, R. H., Jr. 458, 480
Foltz, E. L. 426, 433
Forbes, A. 427
Forbes, J. C. 448
Ford, R. T. 475
Forman, C. 483
Forney, R. B. 481
Forsham, P. H. 420, 456
Forssander, C. A. 420
Foster, R. A. C. 421
Foster, R. H. K. 463
Foster, G. L. 455
Foulks, J. G. 428
Fowle, A. C. 424
Fox, C. L., Jr. 439, 443
Fox, J. P. 492
Fox, S. W. 458
Fraenkel-Conrat, H. 457
Fraenkel, G. 489
Franklin, A. L. 447
Franklin, L. 425, 431, 463
Francis, T., Jr. 492
Frantz, I. D., Jr. 458
Fraser, A. MacL. 452
Frawley, T. F. 433
Frawley, J. P. 467
Frazier, L. E. 483
Free, A. H. 489
Free, H. M. 489
Frederick, J. N. 440
Fredericq, E. 450
Frederickson, E. L. 475
Freedberg, A. S. 429
Freedman, A. M. 418
Freek, J. A. 452
Freeman, B. B. 439
Freeman, S. 443, 454
Freer, R. M. 459
Freinkel, N. 434
Freund, J. 493
Frey, J. S. 421
Freyburger, W. A. 466
Friedgood, C. E. 452
Friedgood, H. B. 443
Frieden, E. 454
Friedman, M. H. F. 437, 446
Friedman, A. 443
Friedman, M. 423
Friend, F. 433
Friedkin, M. E. 450
Friedlander, H. D. 433
Frisell, W. R. 448
Frost, D. V. 485
Fukushima, D. K. 453
Fuller, J. 483
Fulton, J K. 493
Fung, F.-H. 486
Furchgott, R. F. 421
Fuyat, H. N. 473

G

Gaebler, P. H. 453
Gage, T. B. 472
Gajewski, J. E. 474
Galdston, M. 419
Galeski, B. 442
Gant, V. A. 465
Gantt, W. H. 432, 443
Ganz, A. 464
Garcia, J. P. 427
Gardner, D. E. 468
Gardner, E. 427
Gardner, G. M. 478
Gardner, L. I. 453
Garst, J. B. 443
Garvin, J. S. 430
Gaskill, C. J. 483, 484
Gasteiger, E. 421
Gates, A. A. 481
Gaudino, M. 431, 434
Gauer, O. 428
Gaunt, R. 428, 443
Gay, J. 427
Geduldig, R. 486
Geiger, A. 435
Geiling, E. M. K. 466, 471
Geiman, Q. M. 451
Gelfan, S. 427
Gellhorn, E. 427, 432
Gemmel, J. 467
Gemmill, C. L. 452
Geohegan, W. A. 435
Gerard, R. W. 435, 439, 443
Geren, W. D. 456
Gerheim, E. B. 435
German, H. L. 487, 489
Gernandt, B. E. 427
Gershbein, L. L. 439
Gersten, J. W. 433
Gesell, R. 420, 421
Gey, G. O. 479
Ghosh, D. 451
Giarman, N. J. 477
Gibson, J. G. 432, 439
Gierlach, Z. S. 429
Gilman, A. 428
Gilman, L. 460
Gimbel, N. S. 431, 486
Gingras, R. 441
Girling, F. 431
Gjessing, E. C. 452
Glarborg, E. 432
Glasser, O. 444
Glazko, A. J. 465
Glendening, M. B. 436
Glick, D. 457
Glickman, N. 436
Goble, F. C. 490
Goddard, J. W. 487
Goebbel, W. 483
Goebel, W. F. 491
Goetzl, F. R. 441
Gold, H. 467
Goldberg, B. 421, 422
Golden, F. 460
Goldensohn, E. S. 422
Goldin, A. 421, 422
Goldsmith, G. A. 485, 488, 489
Goldstein, H. 425
Goldstein, N. P. 455
Golud, E. F. 460
Goldwater, L. J. 489
Good, C. H. 430

Good, R. A.....480, 483
Good, T. A..... 480
Goodale, W. T..... 430
Goodell, H.....424, 427
Goranson, E. S..... 438
Gordon, A. S.....420, 423
Gordon, H. A..... 490
Gordon, J. D..... 460
Gordon, W. G..... 450
Goth, A..... 474
Gould, T. C..... 463
Gourley, D. R. H..... 442
Gow, R. C..... 428
Gowdey, C. W..... 467
Graham, D. T..... 424
Graham, J. B..... 482
Gram, M. R.....485, 488
Grandage, A.....464, 472
Grant, N. H..... 457
Grant, R.....421, 436
Grant, Rhoda,..... 442
Grant, R. L..... 472
Grant, W. C..... 432
Gray, E. H..... 435
Gray, F..... 467
Gray, J. L..... 448
Gray, M. G..... 473
Greco, A..... 458
Green, D. M.....444, 467
Green, H. D.....431, 463, 464
Green, R. A..... 473
Green, S..... 494
Greenberg, D. M.....459, 488
Greenberg, L. D..... 449
Greenberg, R..... 421
Greene, K. V..... 435
Greenhalgh, M. D..... 421
Greenman, J. H..... 429
Greenman, L..... 428
Greenstein, J. P..... 458
Greenwald, I..... 490
Gregersen, M. I..... 437
Greig, M. E..... 466
Greiner, T..... 467
Griffin, G. E..... 453
Griffith, L. E..... 453
Grimson, K. S..... 472
Grindlay, J. H..... 483
Grodins, F. S..... 422
Grollman, A..... 457
Gross, E. G..... 477
Gross, J..... 429
Grossbert, A..... 438
Grossman, B. J.....435, 439
Grossman, J.....423, 425
Grossman, M. I.
424, 437, 439, 442
Grossman, W. I..... 455
Grover, R. F..... 422
Grover, R. F..... 425
Groves, M. L..... 450
Gruber, C. M.....469, 470
Gruenstein, M..... 421
Gruhzit, C. C..... 469
Grukzkit, O. M..... 464
Grundfest, H..... 430
Guest, M. M..... 436
Guerrant, N. B..... 486
Guevara, R..... 467
Guild, R..... 421
Gunsalus, F..... 452
Gurin, S.....456, 447
Guterman, H. S..... 423
Gutierrez-Noriega, C..... 470
Gutman, A. B..... 456
Gutmann, H. R..... 454
Gyorgy, P.....449, 489

H

Haag, H. B.....473, 477
Hack, M. H..... 423
Hackel, D. B..... 430
Haddy, F. J.....431, 434
Hagan, E. C.....473, 475, 479
Hagan, G. C..... 489
Hagerty, R. F..... 439
Haig, C..... 444
Haines, W. J..... 453
Hakanson, E. Y..... 457
Hales, H. B..... 491
Hall, L..... 489
Hall, L. C..... 457
Hall, P. W..... 431
Hall, R. H..... 465
Hall, V. E..... 421
Hall, W. K..... 485
Halpert, B..... 484
Hamilton, W. F..... 425
Hamilton, W. F., Jr...420, 425
Hammerstrom, R. N..... 477
Hammond, M..... 426
Hampson, J. L...418, 421, 444
Hampton, J. K., Jr..... 439
Handelsman, J..... 426
Handley, C. A..... 471
Hankes, L. V..... 485
Hanna, C. B..... 471
Hansen, E. L.....474, 477
Hanson, H. T..... 458
Hanzlik, P. J..... 478
Happ, W. P..... 439
Hardenbergh, E..... 441
Hardy, J. D..... 432
Hardt, L. L..... 468
Hardy, J. D.....427, 453
Hare, K..... 425
Hare, R. S..... 425
Hare, W. V..... 479
Harger, R. N.....476, 481
Harkness, W. D..... 475
Harlin, H. A..... 489
Harman, J. W..... 483
Harmon, J. B..... 472
Harper, A. E..... 447
Harris, A. S.....420, 444
Harris, F. B..... 472
Harris, J..... 425
Harris, P. N..... 481
Harris, R..... 440
Harris, R. S.....433, 485, 488
Harris, S..... 493
Harris, T. N..... 493
Harrison, H. C..... 444
Harrison, H. E..... 444
Harroun, J. E..... 485
Hart, A. M..... 491
Hart, E. R..... 469
Hart, J. M..... 443
Hartley, L. J..... 482
Hartiala, K..... 442
Hartline, H. K..... 427
Hartman, A. M..... 451
Hartman, F. W.....425, 480
Hartroft, W. S.....442, 489
Hartwell, J. L..... 479
Haskins, D..... 439
Hass, G. M.....479, 480
Hassert, G.L.,Jr..... 470
Hassinen, J. B..... 489
Hastings, A. B..... 449
Hastings, N..... 435
Hatcher, J. D..... 435
Haterius, H. O..... 444
Haurowitz, F.,..... 491
Hawkins, J. E., Jr..... 464
Hawley, E. E..... 488
Hawley, J. G..... 425
Hayes, J. F..... 424
Hayes, R.....423, 487
Haymaker, W..... 438
Haynes, F. W..... 420
Hazleton, L. W..... 470
Hearon, J. Z..... 435
Hecht, L..... 448
Hechter, O..... 420
Hedal, E. F..... 423
Hegnauer, A. H..... 444
Hegsted, D. M..... 488
Heidelberger, M.....450, 491
Heilbrunn, L. V..... 435
Heino, H. E..... 479
Hejtmancik, M. R....420, 471
Heller, C. G..... 426
Hellerman, L..... 448
Hellerman, R. C..... 470
Hellman, L.....420, 425, 444
Helmold, R. J..... 492
Helpern, M. M..... 466
Henderson, F. G.....466, 476
Henderson, L. M..... 485
Henderson, R.....433, 485
Henle, W..... 493
Henneman, E.....424, 470
Henry, J. P..... 428
Henschel, A.....433, 437
Hensen, J. P..... 472
Hepler, O. E..... 484
Heppel, L. A..... 451
Heppleston, A. G..... 480
Herrick, J. F..... 432
Herring, D. A..... 470
Herrmann, G. R.....444, 471
Herrold, E. A..... 466
Heston, W. E..... 479
Hestrin, S.....423, 457
Hertzman, A. B..... 432
Hiatt, E. P..... 472
Hiestand, W. A..... 445
Higashi, A.....475, 477
Higgins, H..... 459
Hightower, N. C..... 437
Hill, C. H..... 447
Hill, D..... 485
Hill, E..... 448
Hill, H. H., Jr..... 486
Hill, J. E.....465, 477
Hill, R. M.....418, 449
Hill, T..... 483
Hilleman, M. R..... 492
Hilliard, I. M..... 419
Hilton, J. G.....428, 452
Himmelstein, A..... 434
Himwich, H. E...418, 421, 444
Hind, J. E..... 418
Hine, C. H..... 476
Hines, E. B..... 429
Hinkle, L. E., Jr..... 438
Hipp, N. J..... 450
Hirsch, A. K..... 457
Hirschmann, F. B..... 458
Hirschmann, H..... 458
Hirshfield, A..... 456
Hitchcock, F. A..419, 427, 428
Hitchings, G. H..... 446
Hodge, H. C.....465, 473
Hodges, R. E..... 431
Hodgkin, A. L..... 436
Hoff, E. C..... 439
Hoff, H. E.....422, 437
Hoffman, C. E..... 447
Hoffmaster, T..... 441
Hogan, A. G..... 487
Hogben, C. A. M..... 428
Hogg, L..... 481
Holbrook, P..... 483
Holder, M..... 434
Holland, O. L..... 466
Holland, W. C..... 466
Hollander, F.....442, 444
Holman, R. L.....481, 484
Holt, L. E..... 469
Holt, L. E., Jr..... 455
Holtkamp, D. E.....418, 449
Hoobler, S. W.....431, 440
Hooper, I. R..... 451
Hoover, C..... 485
Hoppe, J. O.....468, 474
Hopper, J., Jr.....443, 445
Hoppert, C. A..... 489
Hopps, H. C.....481, 494
Horecker, B. L..... 451
Horsfall, F. L., Jr..... 418
Hotchkiss, R. D..... 459
Horvath, S. M..... 433
Houck, C. R.....425, 444
Hove, E. L.....486, 490
Howe, H. A..... 492
Howard, F. A..... 434
Howell, S. F..... 451
Howie, D. L..... 473
Horwitz, O..... 422
Howland, J. W.....465, 481
Huddlestun, B..... 441
Hudson, P. B..... 426
Hudyma, G. M..... 465
Huf, E. G..... 442
Huff, J. W..... 487
Huffman, M. N..... 457
Huggins, R. A..... 471
Hulpieu, H. R....470, 474, 476
Hummel, J. P..... 451
Humphrey, J..... 460
Humphreys, E. M..... 483
Hundley, J. M..... 483
Hunter, F. E., Jr..... 450
Hunter, F. R..... 424
Hunter, J..... 420
Hunter, S..... 449
Hurn, M..... 436
Hursh, J. B..... 481
Huseby, R. A..... 459
Hutchings, B. L..... 447
Hutt, B. K..... 433
Hyde, J..... 427

I

Ingraham, R. C..... 422
Inouye, T..... 436
Ipsen, J., Jr..... 463
Irish, U..... 457
Irvin, E. M..... 450

Irvin, J. L. 450
Isaacs, R. 480
Itano, H. A. 460
Ivy, A. C. 438, 439, 442
Iwamoto, H. K. 472
Izzo, M. J. 480

J

Jacobs, J. 432
Jacobs, M. H. 444
Jaffe, E. R. 482
Jagannathan, V. 457
James, M. F. 487
Jandorf, B. J. 473
Janes, R. G. 441
Jang, R. 458
Janowitz, H. D. 424
Jansen, E. F. 458
Jaques, L. B. 435, 436, 445
Jasper, D. E. 434
Jasper, W. H. 440
Jefferson, N. C. 437
Jeffries, H. 492
Jelinek, V. C. 464
Jenerick, H. 435
Jenney, E. H. 469
Jennings, R. 479
Jensen, H. 448
Jetter, W. W. 468, 477
Jochim, K. E. 430
Joffe, M. 428
Johnson, A. C. 448, 461
Johnson, A. D. 467
Johnson, A. F. 453
Johnson, B. C. 487
Johnson, C. D. 455
Johnson, D. L. 451
Johnson, F. H. 421
Johnson, J. E. 478
Johnson, R. E. 488
Johnson, R. H. 453
Johnson, R. P. 430
Johnson, S. A. 440
Johnson, S. R. 489
Johnson, W. H. 460
Johnston, F. A. 489
Johnston, T. L. 483
Jones, R., Jr. 474
Joseph, N. R. 451
Jukes, T. H. 447
Jung, F. T. 484
Jungck, E. C. 426

K

Kaada, B. R. 435
Kabat, E. A. 455
Kadota, K. 433, 446
Kahn, A. J. 442
Kahn, J. B., Jr. 463
Kahn, R. L. 493
Kalfayan, B. 492
Kalnitsky, G. 449
Kandel, A. 468
Kane, G. 489
Kaplan, A. 470
Kaplan, E. 451
Kaplan, N. O. 448
Karandikar, G. 429
Karczmar, A. G. 469, 473, 477
Kashiwagi, C. 492
Katsh, G. F. 420
Katz, L. N. 440, 422
Katzman, P. A. 457
Kauffmann, G. 491
Kaulbersz, J. 438, 445
Kaunitz, H. 429
Kay, F. A. 460
Kearney, E. B. 448
Keasling, H. H. 475
Keating, J. W. 465
Keating, R. P. 431
Keeton, R. W. 436
Keith, C. K. 447, 487
Kell, J. F., Jr. 435
Keller, H. M. 491
Kelley, B. 452
Kelley, V. C. 483
Kellner, A. 484
Kellogg, W. L. 485
Kelly, A. R. 463
Kelly, M. G. 468
Kelsey, F. E. 470
Kemberling, S. R. 427
Kemmerer, A. R. 485
Kemph, J. P. 427
Kendall, F. E. 448
Kenfield, W. J. 437, 440
Kennedy, E. P. 454
Kennedy, T. J., Jr. 428
Kensler, C. J. 469, 477
Kent, L. J. 485
Kepler, E. J. 457
Kerwin, J. 466
Keston, A. S. 459, 461
Kety, S. S. 428
Keys, A. 418, 426, 431, 433, 437
Kiang, S. P. 420, 443
Kidd, J. G. 483, 492
Kiely, C. E., Jr. 429
Kik, M. C. 490
Kilian, D. J. 434
Kim, K. S. 442
Kimura, T. E. 471
King, A. L. 422
King, C. G. 489
King, E. E. 471
King, E. Q. 493
King, J. T. 426
King, T. O. 477
Kingsland, N. 486
Kinney, V. M. 484
Kirschbaum, A. 483
Kirschbaum, H. 418
Kleiber, M. 434
Kleinschmidt, W. J. 491
Kline, D. L. 433
Klotz, I. M. 461
Klug, H. L. 476
Kluver, H. 444
Knight, D. R. 489
Knott, E. M. 487
Knox, W. E. 455
Knutson, J. 422, 432
Knutson, J. R. 425
Kobrak, H. G. 418
Kochakian, C. D. 458
Koehler, A. E. 448
Koff, A. K. 439
Kokernot, R. H. 420, 444
Komarov, S. A. 421, 437, 438
Koppanyi, T. 469, 477
Koretsky, I. B. 426
Korey, S. R. 442
Korkes, S. 454
Kornberg, A. 451
Korr, I. M. 435, 444
Koschnitzke, H. 434
Koshland, M. 494
Kosman, A. J. 440
Kottke, F. J. 440
Kozloff, L. M. 459
Kraatz, C. P. 469, 470
Krakower, C. A. 479
Kramer, B. 486
Kramer, H. 420
Kramer, K. 422, 428
Krantz, J. C., Jr. 471, 472, 473
Kraus, L. M. 423
Kraus, S. D. 444
Krause, R. F. 486
Kraybill, H. R. 475
Krebs, A. T. 429
Krebs, E. G. 447
Krebs, J. 468
Krehl, W. A. 488
Krider, J. L. 487
Krider, M. M. 486
Krieger, H. P. 424, 427
Krop, S. 469
Krueger, H. 442
Kruse, T. K. T. 432
Krzywicki, H. 488
Kubacki, V. 458
Kubicek, W. G. 440
Kueter, K. E. 474
Kuizenga, M. H. 453
Kun, E. 467, 470
Kuna, M. 454
Kuna, S. 464
Kunkel, H. O. 458
Kuntz, E. 442
Kuramoto, K. 423
Kunze, F. 465, 467, 476
Kusserow, G. W. 471
Kuzell, W. C. 478
Kydd, G. H., III 419
Kyker, G. C. 450, 461

L

LaBelle, A. 472
LaBelle, C. W. 465
Ladd, A. T. 484
Ladd, M. 425
LaFrance, L. J. 465
Laki, K. 439
Lam, C. R. 425
Lamb, A. R. 457
Lambert, J. J. 467
Lambertsen, C. J. 419
Lampen, J. O. 456
Lamport, H. 431
Landowne, M. 445
Lands, A. M. 463
Landwehr, G. 439
Landy, M. 493
Lane, A. 437
Lang, E. H. 468
Lang, L. P. 422, 429
Lange, K. 434
Lange, W. 488
Lanley, J. 463
Lanni, F. 493
Lanphier, E. H. 466
Lardy, H. A. 451, 454
Larsen, E. M. 444
Larson, P. S. 473, 477
Laskin, S. 465
Laskowski, M. 458
Last, J. H. 466
Laug, E. P. 465, 467, 476
Laurent, A. M. 492
Lauson, H. D. 431
Lawrence, B. J., Jr. 464
Lawton, R. W. 422
Layton, W. M., Jr. 453
Leake, C. D. 477
Leary, J. S. 471
Leathem, J. H. 429, 444
Leblond, C. P. 429
Leder, I. G. 449
Leduc, E. H. 433
Lee, A. J. 445
Lee, K. S. 464
Lee, R. E. 435
Lee, R. E. 463
Leese, C. E. 435
LeFevre, M. W. 444
Lehman, A. J. 470
Lehman, R. A. 471
Lehmann, G. 474, 490
Lehmann, J. H. 467
Lehninger, A. L. 450, 454
Lehr, D. 473, 477
Leimdorfer, A. 472
Lein, M. 455
Leiter, L. 423, 425
Lepeschkin, E. 429, 471
Lepkovsky, S. 454
Lepp, E. 436
Lerman, L. 491
Lerner, A. B. 448
Lesser, B. 419
Leverton, R. M. 485, 488
Levey, S. 485
Levin, L. 458
Levine, C. 448
Levine, J. 493
Levine, L. 491
Levine, M. D. 458
Levine, R. 437, 441, 443, 446
Levine, R. B. 430
Levine, V. E. 461
Levitas, N. 461
Levitt, M. F. 431, 434
Levy, B. B. 448
Levy, M. 459, 461
Lewis, H. B. 448
Lewis, J. H. 435
Lewis, J. R. 466
Lewis, L. A. 441
Li, C. H. 450, 461
Libet, B. 435, 443
Lieberman, S. 453
Lief, P. A. 463
Lienke, R. 453
Lifson, N. 438
Light, A. 468
Lillie, R. 420
Lincoln, R. E. 449
Lindberg, O. 451
Lindeman, V. F. 443
Ling, G. 439, 444
Linkswiler, H. 486
Lipmann, F. 452
Lisco, H. 481
Lissner, H. R. 424
Litchfield, J. T., Jr. 468
Little, H. N. 449
Little, J. M. 425, 431
Little, R. C. 425
Little, W. J. 431, 440
Littlejohn, J. M. 454

Liu, C. H. 489
Livermore, G., Jr. 439
Lloyd, D. P. C. 430
Loeb, P. C. 477
Loew, E. R. 475
Loewe, S. 477
Loftfield, R. B. 458
Longabaugh, G. M. 440
Longino, F. H. 472
Loomis, W. F. 451
Looney, J. M. 460
Loosli, J. K. 485
Lorber, S. H. 437
Lorber, V. 441
Lotspeich, W. D. 433
Loube, S. D. 441
Louckes, H. 437
Lovelace, F. E. 489
Loveless, M. H. 493
Loew, T. E. 419
Loynes, J. S. 467
Lu, G. 477
Lubinski, H. H. 491
Lucas, C. C. 442
Lucas, G. H. W. 466
Luck, J. M. 457
Luckey, T. D. 490
Ludewig, S. 452
Luduena, F. P. 474
Luft, U. C. 422
Lundberg, W. 439
Lurie, M. B. 480
Lusky, L. M. 467
Lyman, C. M. 451
Lyster, S. C. 441

M

Maas, W. K. 455
Maaske, C. A. 425
Mabee, D. 486
McArthur, J. W. 453
McCawley, E. L. 477
McCay, C. M. 489
McChesney, E. W. 474
McCord, W. M. 460
McCormick, A. 474
McCormick, J. H., Jr. 481
McCormick, M. H. 448
McCouch, G. P. 430
McCullagh, D. R. 456, 457
McCullagh, R. 426
McDermott, W. 492
McDevitt, E. 488
McDonald, R. K. 431
McGhee, R. B. 480
McGill, H. C. 484
McGilvery, R. W. 458
McGinnis, R. C. 492
McGrath, H. 450
Macht, D. I. 441, 444, 464
McHugh, S. L. 475
McIntire, F. C. 475, 482
McIntyre, A. R. 476
MacKay, E. A. 472
McKee, M. 447
McKee, R. W. 451
McKeen, C. L. 435
McKelway, W. P. 467
McKennis, H., Jr. 448
Mackenzie, C. G. 455
Macko, E. 464, 466, 474
MacLachlan, E. A. 453
McLain, P. L. 432
McMeekin, T. L. 450
McNabb, A. R. 448, 461
McNamara, B. P. 470
McNamara, H. 425
McNamara, P. D. 473
McNaughton, R. A. 455
McNulty, W. P., Jr. 488
McOmie, W. A. 477
MacRae, N. 453
Macri, B. P. 491
MacVicar, R. 447
Maddock, C. L. 479
Maddock, W. O. 426
Mader, I. J. 485
Magasanik, B. 459
Magee, D. F. 442
Magnes, J. 430, 435
Magoun, H. W. 427
Maher, F. T. 437
Maher, P. J. 428
Mahoney, D. I. 428
Main, E. R. 481
Maison, G. L. 471
Majnarich, J. J. 447
Makovsky, A. J. 472
Mallov, S. 429
Maloney, A. H. 463
Maloney, J. E. 479
Maloney, J. V., Jr. 441, 445
Malton, S. D. 467
Maluf, N. S. R. 431
Man, E. B. 454
Mandel, E. E. 479
Manery, J. F. 441
Mangun, G. H. 480, 482
Mann, F. D. 436, 482
Mann, J. D. 482
Mannering, G. J. 490
Manning, G. W. 420
Manson, L. A. 456
Marcus, S. 493
Margaria, R. 427
Margolis, L. 476
Mark, L. C. 463
Mark, V. H. 421
Markee, J. E. 440
Markus, G. 419
Marlow, H. W. 453
Marrazzi, A. S. 469, 473
Marsh, D. F. 470
Marsh, G. 442
Marsh, W. H. 456
Marshall, J. 440, 445
Marshall, L. M. 454
Marshall, M. E. 460
Marshall, W. H. 430, 444
Martens, V. L. 460
Martin, C. G. 422
Martin, C. J. 420
Martin, E. E. 428
Martin, R. A. 480
Martin, W. R. 442
Martindale, W. 447
Martino, E. 464
Mason, H. L. 457
Mason, J. W. 492
Mason, M. F. 461
Masson, G. 443
Mateer, F. 428
Mathies, J. C. 452
Matthews, N. L. 461
Mattill, H. A. 456
Mattison, W. 463
Maurer, P. 450
Maver, M. E. 458
Maxwell, I. A. 484
Maxwell, M. H. 423
Maynard, E. A. 465, 475
Maynard, L. A. 485
Mayo, E. L. 463
Mayock, R. L. 422
Mazur, A. 425, 452, 461
Mead, J. 431, 438
Meek, W. J. 444
Mehl, J. W. 460
Meier, R. 455, 477
Meister, A. 449, 458
Meites, J. 488
Mellinger, G. W. 438
Melnick, J. L. 492
Melville, G. 465
Mendel, D. 491
Mendel, J. 455
Mendez, R. 467
Meng, H. C. 441
Menkin, V. 481
Mero, K. 455
Mertz, E. T. 455
Metcalf, B. H. 472
Mettler, F. A. 432
Metz, B. 444
Mezey, K. C. 467
Michel, H. O. 461, 469
Michel, S. L. 438
Michie, A. J. 431
Mickelsen, O. 453
Middleton, S. 419, 444
Millen, H. M. 439
Miller, G. J. 450
Miller, J. H. 431
Miller, L. C. 469
Milne, L. J. 427
Miller, L. L. 459, 461
Miller, O. N. 487
Miller, R. B. 418
Miller, R. C. 489
Miller, T. 476
Miller, Z. B. 468
Mills, M. B. 487
Milman, A. E. 438
Minard, F. N. 458
Misani, F. 490
Modell, W. 467
Moe, G. K. 466, 467, 469, 478
Mohamed, M. S. 442
Mokotoff, R. 423
Molander, D. W. 483
Molitor, H. 464
Monkhouse, F. C. 435
Moore, C. V. 480
Moore, M. 467
Moore, M. E. 488
Morehouse, L. E. 428
Morgan, A. F. 486, 488
Morgan, C. F. 426
Morgan, D. P. 422
Morgan, H. R. 491
Morgan, I. M. 492
Morgan, J. M. 493
Morita, Y. 452
Morlock, C. G. 437
Morris, L. E. 468, 474, 477
Morris, M. 450
Morrison, D. B. 460
Morrison, J. L. 463
Morrison, P. R. 482
Morrow, P. 465
Morse, M. 434
Morse, R. A. 471
Moruzzi, G. 427
Moses, C. 440, 444
Moses, F. E. 479
Motley, H. L. 422, 429
Moulder, P. V. 435, 439
Mountcastle, V. 424
Moxon, A. L. 476
Moyer, J. 419
Mudge, G. H. 428
Muhrer, M. E. 482
Mulay, A. S. 482
Mulinos, M. G. 473
Mulryan, B. J. 481
Muller, O. H. 459
Muntwyler, E. 453
Murphy, F. J. 476
Murphy, Q. R. 444
Murray, O. B., Jr. 443
Musacchia, X. J. 441
Mushett, C. W. 479

N

Nachmansohn, D. 423
Nagyfy, S. F. 477
Nahum, L. H. 429
Naranjo, A. 481
Nardone, R. M. 424
Nasset, E. S. 485
Nastuk, W. L. 439
Natelson, S. 464
Nathanson, I. T. 426
Necheles, H. 437
Neidle, E. A. 424
Neilands, J. B. 459
Nelson, A. A. 465, 467, 468, 473, 479
Nelson, D. 434
Nelson, E. M. 447
Nelson, J. N. 444
Nelson, T. E. 436
Nelson, W. L. 458
Nelson, W. O. 426, 429
Nemir, R. L. 489
Neuman, M. W. 481
Neuman, W. 465
Neuman, W. F. 481
Neumann, A. L. 487
Neurath, H. 458
Neva, F. A. 491
Neville, J. F., Jr. 434
Newman, E. 442
Newman, M. M. 425
Newman, W. 458
Nichol, C. A. 447
Nichol, J. 431
Nicholas, C. H. 440
Nickerson, M. 472, 477
Nims, L. F. 424
Nitchals, R. M. 487
Nixon, D. J. 475
Nizet, A. 480
Noble, R. L. 428, 433
Noell, W. K. 422
Noland, J. L. 489
Noonan, T. R. 433, 459, 481
Norris, E. R. 447
Norris, L. C. 447, 451
Norris, R. F. 489
North, N. 424
Northrup, D. W. 427

Norton, S. 468
Nothacker, W. G. 482
Novikoff, A. B. 418, 455
Nowinski, W. W. 443
Nuetzel, J. A. 441
Nuter, M. K. 477
Nutting, F. 458
Nyman, C. O. 448

O

Ochoa, S. 454
Odell, L. D. 457
Ogden, E. 437, 444
Ogur, M. 459
Ohler, E. A. 440
Ohlson, M. A. 488
Okey, R. 454
Oldham, H. 488
O'Leary, C. M. 484
O'Leary, E. 425, 471
Olmstead, F. 444
Olsen, M. W. 486
Olsen, N. S. 441
Olson, M. E. 440
Olson, R. E. 487, 488
Omachi, A. 438
O'Neill, G. O. 475
Opdyke, D. F. 419, 425
Oppenheimer, M. J. 422
Orahovats, P. D. 429
Orloff, M. J. 466
Orr, M. F. 480
Ortega, L. G. 421, 422
Orten, J. M. 452, 454
Orth, O. S. 468, 470, 474, 478
Ortiz, P. J. 454
O'Rurke, V. M. 475
Osborn, C. M. 428, 445
Osgood, B. G. 440, 445
Ostro, M. 464
Otis, A. B. 422
Ottinger, B. 491
Ottke, R. C. 449
Overman, R. R. 423

P

Pabst, M. E. 453
Packchanian, A. 491
Page, E. 441
Page, E. W. 436
Page, I. H. 437, 441, 445
Page, S. G., Jr. 473
Paine, R. 434
Paine, R. M. 422
Palmes, E. D. 438
Pan, S. C. 423
P'an, S. Y. 429
Pankopf, R. 488
Pappas, A. 461
Pappenheimer, J. R. 431
Papper, E. M. 463
Pardo, E. G. 440
Parente, N. 457
Parrish, L. E. 484
Parry, T. M. 470
Parsloe, C. E. 477
Parsons, C. L. 419
Parsons, H. T. 486
Paschkis, K. E. 420, 429
Patt, H. M. 424, 445, 481
Patterson, T. L. 438, 445
Paul, W. D. 460
Paul, W. J. 487
Pauling, L. 460
Payne, F. E. 443, 445
Payne, J. T. 481
Payne, M. A. 437
Payne, R. W. 420
Pearlman, W. H. 458
Pearsall, H. R. 460
Pearse, H. E. 481
Pearson, F. 457
Pearson, H. E. 459, 461, 492
Pearson, O. H. 449
Pearson, P. B. 449, 489, 490
Pedersen, H. E. 424
Peeler, A. L. 484
Peeler, H. T. 451
Peet, M. M. 431
Peiss, C. N. 421, 424
Pencharz, R. 454
Peoples, S. A. 474
Perkins, J. F., Jr. 439
Perl, E. 445
Perl, E. R. 422
Perlmann, G. E. 491
Perlzweig, W. A. 449
Pessotti, R. L. 469
Petrenco, H. 493
Peters, J. H. 428
Peters, L. 475, 477
Petersen, D. F. 476
Peterson, E. A. 459
Peterson, L. H. 420
Peterson, W. G. 474
Peterson, W. H. 459
Pettengill, A. 465
Pettinga, C. W. 458
Peyser, P. 419
Pfeiffer, C. C. 463, 466, 469
Phares, E. F. 454
Phillips, C. W. 437
Philips, F. S. 473, 480
Pickering, R. W. 428
Pincus, G. 418
Pincus, J. B. 464
Pisanty, J. 467
Pitcairn, D. M. 437
Pitesky, I. 466
Pitts, R. F. 428, 431
Place, E. F. 456
Plaut, G. W. E. 454
Plummer, A. J. 478
Plympton, A. B. 446
Polglase, W. J. 461
Pollack, A. D. 480
Pomerat, C. M. 480
Pontius, R. G. 419, 434
Popper, H. 479, 482, 484
Porter, A. N. 433
Porter, J. R. 474
Porter, J. W. 449
Porter, T. 488
Posternak, T. 457
Potter, V. R. 450
Powell, M. J. 439
Powers, S. 426
Pratt, E. L. 453
Pratt, H. J. 478
Prec, O. 422
Prescott, B. 491
Prescott, J. M. 451
Press, M. 419
Pressotti, R. L. 452
Pribram, K. H. 435
Price, C. W. 493
Price, T. D. 449
Price, V. E. 458
Prickett, C. S. 476
Prince, R. 437
Pritchard, W. H. 419, 426
Probst, G. W. 449
Proctor, C. M. 457
Proffit, B. K. 460
Prouty, L. R. 438
Prystowsky, R. P. 471
Pullman, T. N. 445
Putnam, F. W. 459
Puttuck, S. L. 477

Q

Quick, A. J. 482
Quigley, J. P. 437

R

Raab, W. 429, 471
Rabinowitz, J. C. 451
Rabinowitz, M. 434
Rader, B. 433
Radin, N. S. 459
Radomski, J. L. 473, 475
Rafelson, M. E., Jr. 459
Rahn, H. 419
Raisz, L. G. 425
Ralston, N. P. 434
Ramsey, H. H. 476
Ramsey, R. W. 442
Randall, L. O. 474
Randall, W. A. 493
Randall, W. C. 432
Rappaport, M. 437
Rapport, D. 421
Raska, S. B. 445
Ratke, H. V. 439
Ratliff, C. M. 463
Ratner, S. 452, 461
Rau, G. C. 445
Ravdin, I. S. 482
Reade, B. 488
Rebuck, J. W. 480
Reed, C. I. 451
Reed, E. A. 422
Reed, J. O. 488
Reed, M. A. G. 472
Reed, R. K. 476
Reid, M. E. 487
Reinecke, R. M. 434
Reiner, J. M. 458
Reiner, L. 461, 490
Reinhard, J. J., Jr. 437
Reinhold, J. G. 460
Reiser, R. 490
Reiss, R. A. 467
Reissman, K. R. 480
Reiter, B. C. 481
Remington, J. W. 434
Rennick, B. R. 467
Reutner, T. F. 464
Reyniers, J. A. 490
Reynolds, M. S. 485, 486
Rice, C. E. 491
Rice, E. F. 456
Rice, E. W. 457
Richards, C. H. 432
Richards, R. K. 466, 474, 482
Richardson, A. P. 473
Richardson, L. R. 487
Richert, D. A. 456, 461
Richmond, G. H. 434
Ridout, J. H. 442
Riegel, C. 431, 486
Riesco-McClure, J. S. 427
Rieser, P. 440
Rigas, D. A. 426
Rigdon, R. H. 478, 479, 480
Riggs, D. S. 470
Riggs, T. R. 488
Riker, W. F. 468, 469
Riley, R. F. 470
Riley, R. L. 419, 434
Rinehart, J. F. 450
Ring, G. C. 422
Rinzler, S. H. 467, 474
Rittenberg, D. 449, 459
Robb, J. S. 429
Robbins, M. E. 436
Roberts, J. T. 425
Roberts, K. 431
Roberts, S. 452
Robertson, C. R. 437, 438
Robertson, T. 484
Robertson, W. v. B. 419, 461
Robinson, A. 460
Robinson, C. S. 460, 461
Robinson, F. 430
Robinson, F. J. 474
Robinson, T. W. 428
Rockenmacher, M. 493
Rodbard, S. 437, 440
Roddy, W. N. 478
Rodenow, E. C. 493
Roderuck, C. E. 456
Rodney, G. 457
Rodriquez, A. A. 466
Roe, J. H. 457, 487
Rogers, C. S. 433, 452
Roggenhofer, J. M. 472
Roos, A. 419, 434
Rose, C. S. 449
Rose, W. C. 489
Roseman, S. 457
Rosen, F. 449
Rosen, G. 459
Rosenbaum, S. J. 470
Rosenberg, A. R. 486
Rosenfeld, M. 460
Rosenkrantz, M. 464
Rosenman, R. 422
Rosenow, E. C. 479
Rosenthal, O. 452
Rosenthal, S. R. 480
Rossiter, R. J. 448, 455, 461
Rostenberg, A., Jr. 466
Roth, F. 466, 470
Roth, G. M. 436
Roth, L. W. 482
Rothchild, M. L. 447
Rothenberg, M. A. 421
Rothstein, A. 455, 476
Routh, J. I. 460
Rovenstine, E. A. 463
Rowland, W. E. 485
Rubenstein, L. H. 423
Rubin, A. J. 426, 433
Rubin, B. A. 477
Rubin, S. H. 488
Ruhe, C. H. W. 432
Rupp, J. 429
Rushmer, R. F. 418
Ruskin, A. 478, 480
Russ, S. B. 492
Russell, D. 466, 472

Russell, J. A. 438
Russell, L. B. 481
Russell, W. C. 486
Rusterholz, A. P. 479
Rutledge, E. K. 418
Ryland, K. W. 442

S

Sabin, A. B. 492, 493
Sabine, J. C. 441
Sacher, G. A. 424
Sackis, J. 424
Sacks, J. 441
Sahyun, M. 477
St. George, R. C. C. 449
Sakami, W. 459
Salerno, P. R. 469, 472
Salk, J. E. 492
Salmon, W. D. 486, 487, 490
Salomon, K. 433, 459, 481
Salter, W. T. 467
Saltzstein, H. C. 438, 441, 445
Samaras, S. C. 461
Samuels, A. J. 435
Samuels, L. T. 458
Samuelsen, G. S. 453
Sanadi, D. R. 488
Sanderson, M. H. 435, 439
Sandow, A. 442
Sandweiss, D. J. 438, 441, 445
Sandy, H. R. 485
Sarett, H. P. 485
Sarnoff, S. J. 432, 437, 445
Sartorius, O. W. 431
Sauberlich, H. E. 447
Saunders, F. W. 448
Sawtelle, W. W. 477
Sawyer, C. H. 424
Sayers, G. 420
Sayers, M. A. 420
Schachner, H. G. 429
Schachter, D. 434
Schaefer, A. E. 487
Schaffarzick, R. W. 478
Schaffenburg, C. 443
Schaffner, F. 482
Schayer, R. W. 455
Schein, A. H. 456
Schepartz, B. 447
Schiaffino, S. S. 458
Schick, A. F. 480
Schiller, A. A. 441
Schlaphoff, D. 489
Schlenk, F. 475
Schlesinger, R. S. 468
Schlesinger, R. W. 493
Schmidt, G. 448
Schmidt-Nielsen, B. 438
Schmidt-Nielsen, K. 438
Schmitt, F. O. 418
Schmitt, O. H. 421, 430
Schnabel, T. G., Jr. 420
Schneider, H. A. 489
Schneider, J. J. 457
Schneider, W. C. 450
Schoenbach, E. B. 422
Schoenfeld, R. C. 431
Schoepfle, G. M. 435
Schour, L. 440
Schroeder, H. A. 437
Schroeder, L. J. 485
Schroer, G. 428
Schueler, F. W. 475
Schuhmacher, L. F. 471
Schults, J. E. 467
Schultz, J. 493
Schultze, M. O. 449, 485
Schwab, E. H. 444, 471
Schwartz, I. L. 423, 434
Schwenck, E. 426, 464
Schwerin, P. 447
Schwerma, H. 422
Sciarini, L. 467
Scott, D., Jr. 424
Scott, E. B. 468
Scott, J. C. 422
Scott, J. K. 465
Scott, M. L. 447
Scott, W. W. 426
Scrimshaw, N. S. 483, 488
Scroggie, A. E. 445
Seager, L. D. 464
Seaman, G. R. 424
Sebrell, W. H. 489
Seed, J. C. 472
Seegers, W. H. 482
Seevers, M. H. 467
Segaloff, A. 483, 484
Seidlin, S. M. 454
Seifter, J. 465
Seifter, S. 452
Seki, S. L. 468
Seldin, D. W. 428, 441
Selkurt, E. E. 431
Sellers, E. A. 423
Selye, H. 420
Semmett, W. F. 450
Sennett, L. W. 422, 423
Settlage, P. H. 432
Sewell, M. 423
Sewell, M. B. 488
Shacter, B. 441
Shane, S. J. 467
Shanes, A. M. 421
Shapiro, S. 471
Shaw, J. H. 487, 489
Shay, H. 421, 437, 438
Sheft, B. B. 488
Shelton, D. C. 455
Shemin, D. 455, 459
Sheppard, C. W. 442
Sherman, F. G. 424
Sherman, H. 488
Sherwood, M. B. 449
Shideler, A. M. 494
Shideman, F. E. 463, 474, 478
Shields, H. L. 469
Shils, M. E. 489
Shingleton, W. W. 423
Shirley, R. L. 489
Shock, N. W. 422, 423, 431, 486
Sholes, D. M. 435
Shorr, E. 421, 423, 425, 437, 440, 452, 461
Shoshkes, M. 470
Shrader, R. E. 486
Shuck, C. 487
Shuman, R. 460
Siegfried, D. A. 454
Siegmund, O. H. 463
Siekevitz, P. 459
Silber, R. H. 483
Silverman, T. 470
Simmonds, S. 449
Simon, A. 476
Simonsen, D. H. 451
Simonson, E. 426
Simpson, M. 454
Simpson, Z. A. 482
Sims, J. L. 474
Sims, P. M. 444, 471
Sinclair, R. G. 458
Singal, S. A. 454
Singer, S. J. 450
Singer, T. P. 448
Sirota, J. H. 445
Sizer, I. W. 445, 491
Slanetz, C. A. 429
Slater, I. H. 420, 443
Slaughter, D. 465
Slaunwhite, W. R., Jr. 426
Sleator, W., Jr. 434
Slein, M. W. 457
Sloane, N. H. 451
Smetak, E. M. 455
Smith, A. H. 434, 454, 485
Smith, B. P. 456
Smith, B. W. 457
Smith, C. C. 474, 483
Smith, D. E. 424, 481
Smith, E. L. 452, 458, 461
Smith, F. A. 465, 468
Smith, G. N. 464
Smith, H. L. 418
Smith, H. W. 473
Smith, J. 466
Smith, J. R. 434
Smith, M. I. 468
Smith, N. 466
Smith, P. K. 468
Smith, T. C. 423
Smyth, C. J. 485
Snell, E. E. 451, 455, 486
Snell, J. F. 459
Snider, R. S. 432
Snoddy, W. T. 484
Snyder, F. F. 445
Snyder, R. 439
Snyder, R. H. 486
Snyderman, S. E. 489
Sobel, A. E. 453, 486
Sober, H. A. 448
Solomon, J. D. 454
Sonnenschein, R. R. 424, 469
Soodak, M. 448
Sorensen, E. 466, 471
Sorof, S. 452
Speigel-Adolf, M. 461
Spencer, F. 426
Spencer, J. N. 422, 464, 470
Spencer, M. P. 431
Sperry, W. M. 448
Spicer, S. S. 441
Spiegel, E. A. 427, 445
Spiegl, C. J. 465
Spitzer, A. 475
Spooner, M. 439
Sprague, G. 465
Sprague, G. F. 465
Sproul, M. 475
Sprunt, D. H. 479, 493
Stacy, I. B. 492
Stamler, J. 440
Standley, E. T. 482
Stannard, J. N. 419
Stare, F. J. 487, 488
Starr, I. 422
Stavitsky, A. B. 493
Stavitsky, R. 493
Stavraky, G. W. 430, 433
Stebbins, R. B. 479
Steel, J. M. 448
Steele, B. F. 485
Steele, J. M. 434
Steelman, S. L. 450
Steenbock, H. 486
Stefanini, M. 461, 482
Steffee, C. H. 483
Steiger, W. A. 426
Steigmann, F. 468, 482, 484
Steinberg, B. 480
Steinhardt, J. 450
Steinhaus, A. H. 475
Steinhaus, J. E. 474
Steinkamp, R. 488
Stekol, J. A. 454
Stephens, C. A. 485
Sternberg, W. H. 483, 484
Sterner, J. H. 475
Stettin, DeW. 456
Stewart, H. M. 485
Stewart, J. M. 480
Stewart, M. 435
Stewart, R. 431
Stewart, W. B. 430, 480
Stewart, W. E. 479
Stickney, C. 427
Stigman, S. 441
Still, J. L. 454, 461
Stimmel, B. F. 457
Stockell, A. 488
Stoerk, H. C. 483
Stohlman, E. F. 468
Stokinger, H. E. 465
Stokstad, E. L. R. 447
Stone, C. A. 475
Stone, H. 420
Storvick, C. A. 487
Stotz, E. 449
Stout, C. 444
Stow, R. W. 433
Straube, R. L. 481
Strauss, L. 481
Strecker, H. 448
Strength, D. R. 487
Strickler, N. 448
Strifert, J. 458
Strong, F. M. 459
Strong, F. M. 454
Stroud, M. 426
Stullden, D. E. 445
Stutzman, F. L. 434
Stutzman, J. W. 471
Suárez, R. M. 487
Suckle, H. M. 432, 470
Sugioka, K. 434
Sullivan, C. 432
Sullivan, M. X. 458
Summerson, W. H. 448, 492
Sunahara, F. 430, 435
Sunderman, F. W. 462, 475
Sung, C. 466
Sure, B. 486
Surgenor, D. M. 460
Susca, L. A. 445
Susea, L. A. 427
Sutherland, E. 457
Sutherland, V. C. 466, 476
Svec, M. H. 445
Svihla, G. 424
Swann, H., II 425
Swanson, A. 457

Swanson, M. A. 457
Swanson, P. 485
Swarm, R. L. 460
Swell, L. 461
Swenson, R. E. 480
Swift, M. N. 481
Swift, M. N. 445
Swinyard, E. A. 466
Sydenstricker, V. P. 454, 485
Symeonidis, A. 484
Szanto, P. B. 482
Szego, C. M. 433

T

Tabor, H. 448
Tacket, H. S. 445
Tager, M. 491
Talesnik, J. 419, 444
Taliaferro, L. G. 493
Taliaferro, W. H. 493
Tarail, R. 428, 441
Tarver, H. 454
Tatum, E. L. 418, 449
Taube, H. 471
Tauber, H. 458
Taylor, B. E. 422, 425, 432
Taylor, C. B. 479
Taylor, E. 436
Taylor, H. D. 451
Taylor, H. L. 431, 433, 437
Taylor, H. W., Jr. 474
Taylor, J. D. 466
Taylor, J. F. 460
Taylor, M. W. 486
Taylor, N. B. G. 428
Taylor, R. D. 445
Taylor, R. M. 435
Teague, R. S. 469
Teasdall, R. D. 430
Teply, L. J. 459
Tepperman, H. M. 423
Tepperman, J. 423
Terry, M. L. 453
Terry, R. 483
Teslik, H. 451
Teuber, H. L. 424
Thannhauser, S. J. 448
Thauer, R. 436
Therien, M. 438
Thienes, C. H. 463
Thiersch, J. B. 473, 480
Thomas, J. 478
Thomas, J. E. 442
Thomas, J. Y. 448
Thomas, R. K. 470
Thomas, T. B. 484
Thomas, W. D. 431
Thomason, M. J. 488
Thompson, C. R. 473
Thompson, P. E. 464
Thompson, R. T. 479
Thorn, G. W. 420, 432
Thornton, E. R. 440, 444
Thorp, W. T. S. 468
Tieman, J. W. 486
Tipton, S. R. 423
Tishkoff, G. H. 451, 459
Tislow, R. 426, 464, 472
Tkacz, L. P. 442
Tobey, H. L. 488
Tobias, J. M. 443
Tocantins, L. M. 439
Tohertkoff, V. 434
Tolksdorf, S. 426, 456, 457
Toman, J. E. P. 421, 466
Tomarelli, R. M. 489
Tompkins, D. 434
Tompsett, R. 492
Tonhazy, N. E. 454
Toolan, H. W. 483
Toole, J. F. 468
Torda, C. 424, 445
Toribara, T. Y. 465
Tornaben, J. 468
Totter, J. R. 447, 487
Tourtellotte, W. W. 468
Tove, S. B. 487
Towbin, E. J. 436
Towery, B. T. 453
Trager, W. 480
Trapold, J. H. 466
Travell, J. 467, 474
Travis, B. L. 445
Treadwell, C. R. 455, 461
Tree, H. G. 465
Trexler, P. C. 490
Tripi, H. B. 478
Tripp, E. 444
Truant, A. P. 468
Tschirgi, R. D. 435
Tucker, J. M. 479
Tullar, P. E. 478
Tullis, J. L. 481
Turck, L. 491
Tureman, J. R. 463
Turman, W. G. 429
Tuttle, W. W. 439
Tyler, D. B. 435
Tyree, E. B. 445, 481

U

Udenfriend, S. 459, 461
Ullyot, G. E. 421, 466, 474
Umbreit, W. W. 454
Underwood, A. L. 465
Ungar, G. 494
Unna, K. 470
Unterman, D. 471
Ureles, A. 429
Utter, M. F. 454

V

Vail, E. G. 428
Valentine, F. 457
Valentine, J. D. 456
Vanderlinde, R. 453
Van Dilla, M. 429
van Harreveld, A. 432
Van Liere, E. J. 427
Van Maanen, E. F. 470
Van Middlesworth, L. 429
van Noordwijk, J. C. 435
Van Reen, R. 486
van Wagtendonk, W. J. 451, 462
Vars, H. M. 433, 452, 482
Veiga Salles, J. B. 454
Vennesland, B. 451
Vernon, E. R. 454
Vestal, B. 455
Vestling, C. S. 457
Victor, J. 479, 480
Villarreal, H. 445
Villee, C. A. 449
Virtue, R. W. 475
Vischer, E. 459
Visscher, F. E. 441
Visscher, M. B. 426, 431, 435
Visser, D. W. 455
Vogt, M. 420
Volwiler, W. 483
Vos, B. J. 475

W

Waelsch, H. 435, 447
Wagley, P. F. 491
Wagman, I. H. 427
Wagner, J. C. 479
Wagner, M. 490
Wagner, R. R. 492
Waife, S. O. 494
Wainio, W. W. 482
Weiss, P. 490
Wakerlin, G. E. 440, 445
Wakim, K. G. 433
Walcott, W. W. 437
Wald, G. 449
Waldron, J. M. 442
Walker, H. A. 463, 473
Walker, S. M. 442
Walton, R. P. 419, 471, 478
Wallraff, E. B. 485
Waltz, R. H., Jr. 455
Wang, C. C. 442, 454
Wang, K. J. 442
Wang, S. C. 429, 435
Ward, H. P. 431
Ward, J. W. 430
Ware, A. G. 482
Ware, L. 491
Warner, R. C. 456
Warren, J. 492
Warren, M. R. 466
Wartman, W. B. 479
Wase, A. W. 482
Waterhouse, C. 465
Waters, L. L. 479
Waters, R. M. 474
Wattenberg, L. W. 457
Watts, D. T. 474, 478
Waud, R. A. 467
Waugh, D. F. 450
Wawzonek, S. 429
Wax, H. 458
Wax, J. 470
Way, E. L. 466, 478
Wayne, H. 431, 463
Webb, H. M. 443
Webb, M. 448
Weber, W. W. 461
Webster, J. H. 483
Weeks, J. R. 468
Wegria, R. 431
Weinbach, E. C. 448
Weiner, D. 434
Weiner, M. 471
Weisberg, H. F. 441, 443
Weiss, C. 493
Weiss, K. 454
Weiss, E. 481
Weiss, P. 461
Weiss, W. 464
Welch, A. D. 447
Welch, H. 493
Welsh, J. H. 424
Wells, B. 480
Wender, S. H. 472
Wennesland, R. 421
Werner, A. S. 458
Werner, A. Y. 427
Werner, H. W. 472, 473
Wescoe, W. C. 469
Wescott, F. 465
Wesson, L. G., Jr. 425
West, C. D. 458
West, H. D. 454
West, M. 486
Westerfeld, W. W. 453, 456, 461
Westerman, B. D. 489
Westfall, B. A. 463
Weston, R. E. 423, 425
Wetter, L. R. 491
Wheeler, A. H. 493
Wheeler, L. M. 457
Wheeler, P. 441
Whieldon, J. A. 432
White, A. 433, 452
White, C. 420
White, F. B. 475
White, F. M. 445
White, H. L. 434
White, R. B. 443
Whitehead, R. W. 422, 464, 470
Whitehorn, W. V. 422
Whitley, R. W. 457
Whitney, D. M. 464
Whittenberger, J. L. 432, 445
Wiercinski, F. J. 418
Wiersma, C. A. G. 421
Wight, K. 454
Wilber, C. G. 441, 445
Wilcox, P. H. 445
Wilde, W. S. 433, 445
Wilder, O. H. M. 475
Wilhelmi, A. E. 449
Wilkins, D. S. 478
Wilkins, R. J. 470
Williams, A. H. 431
Williams, H. H. 456, 485
Williams, H. L. 463, 466
Williams, M. 440
Williams, R. H. 453
Williams, W. L. 446, 482
Williamson, J. H. 464
Willis, A. 421
Wills, J. H. 464
Wilson, D. L. 441
Wilson, D. W. 456
Wilson, H. 453
Wilson, J. W. 433
Wilson, M. 439
Wilson, M F. 447
Wingard, C. 469
Winnick, T. 459
Winslow, J. A. 425
Winsten, W. A. 447
Winter, C. A. 467, 475
Winter, H. A. 437
Wintrobe, M. M. 480
Winzler, R. J. 454, 459, 461, 492
Wirts, C. W. 446
Wissler, R. W. 482, 483
Witebsky, E. 491
Witham, A. C. 428
Witter, R. F. 449
Wohl, M. G. 494
Wolbach, S. B. 479
Wolf, A. 455
Wolf, A. V. 428
Wolf, L. M. 465
Wolff, H. G. 424, 427, 445
Wolfson, W. Q. 433, 446

Wollenberger, A..... 467
Wong, E..... 455
Wood, E. H.....422, 425, 432
Wood, H. G..... 448
Wood, J. L.....453, 454, 486
Woodard, G.....468, 473, 475
Woodard, P. H..... 494
Woodard, M..... 473
Woodbury, D. M..... 420
Woodbury, J. W..... 444
Woodbury, L. A.....432, 477
Woodbury, R. A..466, 469, 471
Woods, H. L..... 480
Wooley, J. G..... 441
Woolridge, R. L..... 483
Woolsey, C. N..... 432
Wooster, H.....462, 475
Worrell, C..... 464
Wright, E. B..... 430
Wright, H. N..... 474
Wulsen, R. M..... 481
Wulzen, R..... 446
Wurtz, E..... 487
Wycis, H. T..... 461
Wyman, L..... 491

Y

Yamamoto, R. S..... 455
Yen, C. K..... 480
Yiengst, M. J..... 486
Yonkman, F. F..... 477
You, S. S..... 423
Youmans, W. B.....418, 437
Young, D. B..... 443
Young, G..... 482
Young, L. E..... 480
Young, W. C..... 426
Yu, T. F..... 456

Z

Zabin, I..... 449
Zaffarone, A..... 451
Zager, L. L..... 477
Zaiser, E. M..... 450
Zanetti, M. E..... 487
Zarrow, M. X..... 426
Zavertnik, J. J..... 439
Zbarsky, S. H..... 453
Zeller, E. A.....455, 462
Zerbe, J. W..... 452
Ziegler, R. F..... 425
Zill, L. P..... 462
Zimmerman, W. J..... 453
Zizmer. N..... 454
Zucker, M. B..... 434
Zweifach, B. W.
421, 423, 435, 440